DETERMINING LANDSCAPE CONNECTIVITY THROUGH AMPHIBIAN ABUNDANCE, COMMUNITY COMPOSITION, AND GENE FLOW

by

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(Under the Direction of Jeffrey Hepinstall-Cymerman)

ABSTRACT

The composition, configuration, and connectivity of landscapes influence regional ecology. Distinguishing landscape and local effects is difficult, and important effects may be masked, misinterpreted, or ignored if studies are too general. I studied the landscape connectivity of two species of frogs in geographically isolated wetlands (GIWs) on a portion of the Dougherty Plain in southwestern Georgia. I examined the effects of landscape features on amphibian abundance, amphibian community composition, and gene flow of the southern leopard frog (*Lithobates sphenocephalus*) and the southern cricket frog (*Acris gryllus*). Land-use/ land-cover, soil characteristics, and dominant wetland vegetation, have divergent effects on amphibian species and guilds. Percent forest cover and predicted wetlands (based on the occurrence of hydric soils) best explained amphibian abundance and diversity. Genetic differentiation, as measured by F_{ST}, was correlated with Euclidean distance and cost distance for populations of southern cricket frogs, but not for the larger and more vagile southern leopard frogs.

INDEX WORDS: Connectivity; landscape; amphibian; community composition; wetland; geographically isolated wetland; genetic differentiation; gene flow; RADseq; land-use

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INTRODUCTION AND LITERATURE REVIEW

INTRODUCTION

Landscapes are complex systems, and the alignment and interaction of habitat and non-habitat patches, known as *structural connectivity*, strongly influences the interactions among populations and landscapes, known as *functional connectivity*. Functional connectivity allows amphibian movement through landscapes (Revilla et al. 2004, Bender and Fahrig 2005), which in turn determines the ability of organisms to discover new territories, food resources, and reproductive opportunities (Harper et al. 2008) and facilitates gene flow (Coulon et al. 2004). Understanding these patterns and quantifying landscape effects on individual organisms or guilds of organisms can assist scientists and policy-makers in the creation of data-driven best management plans, especially if the goal is conservation, as the dynamics of landscapes influence ecological processes.

Landscapes are far from static. In the wake of climate change and exponential human population growth, habitats are being altered at an unprecedented rate. Habitat loss is the leading cause of species decline and global biodiversity loss (Fahrig 2003, Fischer and Lindenmayer 2007, Didham et al. 2012). The largest driver of habitat loss is human alteration of natural habitats, including deforestation, land-use conversion, and urbanization (Vitousek et al. 1997, Foley et al. 2005). Habitat loss and habitat fragmentation create isolated patches of habitat (Smith et al. 2009, Didham et al. 2012, Haddad et al. 2015), leading to increased rates of extinction and a reduction of biodiversity (Saunders et al. 1991, Fahrig 1997, 2003, Brook et al. 2008). The remaining habitat, specifically the composition and configuration of habitat patches, dictates species persistence.

The effects of habitat conversion are not uniform; species with narrow habitat requirements or who depend on multiple habitats such as amphibians, may be particularly vulnerable to habitat loss and fragmentation (Semlitsch and Bodie 2003, Cushman 2006). Amphibians are in global decline (McCallum 2007). This loss of amphibian species is an intrinsic loss of biodiversity and also results in altered food webs and and potentially cascading effects on ecosystems, as amphibians are highly productive and abundant (Gibbons et al. 2006).

Amphibian distribution, dispersal, and habitat selection are understudied, which impedes the development of data-driven management— especially for imperiled species (i.e. *Ambystoma cingulatum* or *Lithobates capito*). To examine landscape effects within an anthropogenically influenced landscape, I used amphibians as focal taxa, and a portion of the Dougherty Plain as a model ecosystem. The Dougherty Plain physiographic province was historically dominated by the longleaf pine – wiregrass (*Pinus palustris* and *Aristida stricta*) ecosystem and contains abundant limestone-sink geographically isolated wetlands (GIWs). The sandy soils of the region are also suitable for irrigated agriculture, and an estimated 97% of original longleaf pine forest in the region has been converted to agriculture and other anthropogenic land uses (Frost 1993). A shift in agriculture occurred in the 1970's when small dryland farms gave way to large-scale, center-pivot irrigated row crop agriculture. This land-use change fragmented the landscape considerably; however, many embedded GIWs remained even as the wetland vegetation and surrounding uplands were altered (Martin 2010). It is well known that GIWs embedded in longleaf pine forests support considerable diversity of plants (Kirkman et al. 1999), invertebrates

(Battle and Golladay 2002), and amphibians (Smith et al. 2006). Considerably less is known about the diversity of these organisms in GIWs in altered landscapes. To what degree, therefore, does the current and past landscape structure of the Dougherty Plain affect amphibian abundance, community composition, and gene flow? What conclusions can we draw regarding the biological connectivity among GIWs within this agricultural-forest matrix on the Dougherty Plain?

To address these questions, I surveyed amphibians within GIWs on the Dougherty Plain, embedded in a land-use gradient ranging from intact longleaf pine forest to intensive irrigated agriculture. First, I compared the amphibian community composition in wetlands in a forested landscape to the amphibian communities in wetlands in altered landscapes. Next, I created generalized linear models (GLMs) to test the relative explanatory values of surrounding land use/land cover on amphibian abundance within GIWs. I used results of these models to create theoretical least-cost paths and to model landscape resistance. Lastly, I examined the gene flow of two common amphibian species with divergent body sizes and natural histories, *Lithobates sphenocephalus* and *Acris gryllus*, among GIWs. I used a measure of gene flow, the index of fixation (F_{ST})(Ahrens et al. 1990), to both test the resistance model and create a second, corridor-based model to indentify land-use/land-cover which could facilitate or restrict gene flow.

LITERATURE REVIEW

Landscape Connectivity

Landscape connectivity, defined as “the degree to which the landscape facilitates movement among resource patches” (Taylor et al. 1993), is a measure of habitat quality that focuses on the interactions between the patterns of resources on the landscape (i.e., “landscape pattern") and ecological processes. Movement, in this context, can refer to the movement of organisms or any other dynamic ecological processes. This definition encompasses both *structural connectivity*, the physical arrangement of habitat patches and the intervening non-habitat, and *functional connectivity*, the actual movement of organisms between and among patches (Tischendorf and Fahrig 2000). The presence, absence, and spatial distribution of habitat patches and the characteristics of the intervening non-habitat (i.e., the background "matrix") determine the connectivity of the landscape (Tischendorf and Fahrig 2000, Uezu et al. 2005). Connectivity is a critical aspect of ecology, and influences the spread of natural disturbances, infectious diseases, gene flow, propagule dispersal, and metapopulation dynamics (Hanski 1998, Kinlan and Gaines 2003, Coulon et al. 2004, Crowl et al. 2008). Conservation strategies for amphibians include the protection of a variety of wetlands within a matrix of intact upland habitats (Semlitsch and Bodie 1998, Joyal et al. 2001, Liner et al. 2008). The basis for this strategy is to accommodate metapopulations (Marsh and Trenham 2001), where individual wetlands support a larger regional population (Herrmann et al. 2005). Metapopulation theory attempts to explain dynamic patterns of extinction and recolonization among local populations whose interactions collectively support regional populations (Hanski 1998). Many metapopulation models are criticized for discounting the effects of the landscape matrix in its entirety, including considering non-habitat patches within the matrix as homogeneous and unimportant (Tischendorf and Fahrig 2000, Kindlmann and Burel 2008). Anthropogenic alterations to the landscape, including changing land-use and resultant habitat fragmentation, can have a disproportionate effect on those organisms with complex life-history strategies (i.e., aquatic and terrestrial life stages) (Becker et al. 2007), such as amphibians (Cushman 2006).

Amphibian Ecology

Many amphibians have a complex life history strategy. Their gilled, aquatic larvae depend on wetlands and larvae metamorphose to air-breathing juvenile and adult forms that may inhabit terrestrial systems (Duellman and Trueb 1986), although some species have developed adaptations to bypass this ontogenetic shift (Wilbur and Collins 1973, Dodd and Dodd 1976). Frogs account for more than 85% of extant amphibians globally (Duellman and Trueb 1986) and generally have biphasic life cycles. Adult frogs deposit eggs in moist or inundated sites, which hatch into aquatic tadpoles. Tadpoles can occur in high densities within their aquatic breeding habitats and are important components of aquatic food webs (Jenssen 1967, Heyer et al. 1975, Dodd and Dodd 1976). Tadpoles metamorphose into carnivorous adult frogs, which may remain in aquatic environments or seek out uplands for shelter and forage, which allows adult anurans to survive seasonal drying of wetlands without aestivation (Wassersug 1975).

Amphibians occur on every continent except Antarctica (Duellman and Trueb 1986, Duellman 1999, Pimenta et al. 2005). Adult amphibians are susceptible to environmental toxins and desiccation due to their semi-permeable skin and a small surface-to-volume ratio (Duellman 1999), making them more likely to suffer from the effects of urbanization and global climate change, including changes in temperature and precipitation (Walther et al. 2002, Parmesan and Yohe 2003, Pounds et al. 2006). Due to their broad distribution, abundance in certain habitats, importance in connecting food webs, and highly permeable skin (Peacor and Werner 1997, Gawlik 2002, Kats and Ferrer 2003, Colón-Gaud et al. 2009), amphibians have been used as potential bioindicators of environmental health (Carey and Bryant 1995, Collins and Storfer 2003, Niemi and McDonald 2004, Jensen 2008). Amphibians are globally in decline (McCallum

2007), in large part due to habitat loss, degradation, and fragmentation (Brooks et al. 2002, Fahrig 2003, Cushman 2006).

The Southeastern United States has high amphibian diversity (Houlahan et al. 2000), and the Coastal Plain is the most herpetologically diverse region in Georgia (Jensen 2008). Many amphibians in the region are habitat specialists, however, and are thus increasingly imperiled due to habitat fragmentation, expanding agriculture, and urbanization (Semlitsch and Bodie 1998, Smith et al. 2006). The presence, absence, or abundance of amphibians, of selected species or as an assemblage, could therefore be an indicator of habitat suitability of the GIW and the surrounding landscape. Among amphibians, body size is thought to be a predictor of dispersal ability. Small species may be poor dispersers due to smaller energy reserves and an increased risk of water loss related to greater surface area to body size ratio (Mazerolle 2001, Rothermel and Semlitsch 2002), and among amphibians, habitat specialists would be expected to be more affected by changes in land-use than more generalist species (Griffith and Sultan 2012). Amphibian movement patterns and resilience to land-use alteration are difficult to quantify, but are vital to our understanding of amphibian population dynamics— especially in habitats characterized by periodic and sometimes unpredictable drydowns (e.g., GIWs).

Dougherty Plain

My study area is within the Dougherty Plain physiographic region in the Coastal Plain of Georgia, a region characterized by karst topography (Beck and Arden 1983). The Dougherty Plain was once dominated by the longleaf pine (*Pinus palustris*) -wiregrass (*Aristida stricta*) ecosystem (Bragg 2002), which encompassed an estimated 40% of the Coastal Plain in the southeastern United States (Noss et al. 1995). The longleaf pine – wiregrass ecosystem is characterized by an herbaceous, open, diverse understory and sparse pine canopy maintained by frequent fires (Brockway and Lewis 1997), and is home to many endemic or specialist species, especially herpetofauna (Guyer and Bailey 1993).

The hydrogeology of the Dougherty Plain is determined by the underlying Ocala limestone, the dissolution of which has formed sandy substrate and frequent surficial depressions (Kirkman et al. 2000). These surficial depressions may be covered by an impervious or semi-impervious clay lens, which allows precipitation or groundwater to accumulate, forming GIWs (Tiner 2003). GIWs are frequent on the Dougherty Plain, with minimum density estimated at 1.7 wetlands per km^2 (Hendricks and Goodwin 1956) and an average distance of less than 200 m to the nearest neighboring wetland during periods of high water retention (Martin et al. 2012).

The sandy soils that supported the longleaf pine ecosystem and embedded GIWs are also suitable for agriculture (Martin et al. 2012). Forested uplands currently cover ~ 30% of the region, while agricultural uplands cover ~25% (Martin et al. 2012). Unirrigated agriculture has been largely replaced by large-scale, center pivot-agriculture and planted pine since 1968 (Martin 2010). This land-use pattern is also reflected at a larger scale. The expansion of agriculture, silviculture, and urbanization, as well as anthropogenic fire suppression, has reduced longleaf pine ecosystems in the southeastern United States from an estimated 372,311 km^2 to less than 7,500 km^2, or a loss of more than 97% (Frost 1993, Landers et al. 1995). Land-use conversion to agriculture and the corresponding increase in irrigation have altered the hydrology of the region. A large portion (85%) of the water used for irrigation in the Dougherty Plain is drawn from the Upper Floridian Aquifer (Litts et al. 2001), and the surface waters (rivers and wetlands) experience nutrient and sediment run-off from agricultural fields (O’Brien et al. 1998).

Geographically Isolated Wetlands

The seminal definition of wetlands is "lands transitional between terrestrial and aquatic systems where the water table is usually at or near the surface or the land is covered by shallow water" (Cowardin et al. 1979). This encompasses marshes, swamps, floodplains, and peat lands, which account for ~6% of the world's land cover (Meyer and Turner 1992, Gosselink and Maltby 1993, Williams 1993). The U.S. Environmental Protection Agency (EPA) defines wetlands based on the presence of three attributes: hydrophytes (water-dependent vegetation), hydric soils, and the presence of water for long enough to produce hydric soils and characteristic plant communities (Kadlec and Wallace 2008). Unlike many other aquatic environments, wetlands are considered an impediment to agricultural and residential land-use (Blumm and Zaleha 1989). The U.S. Fish and Wildlife Service estimates that half of the wetlands in the United States, or approximately 100 million acres, have been drained (Dahl 1990). Despite public indifference, wetlands support high biodiversity and help sustain healthy water systems (Wienhold and Valk 1989, Tiner et al. 2002, Zedler and Kercher 2005). Government ownership of wetlands or the development of conservation easements surrounding the wetlands is often prohibitively costly, however. This leaves regulatory actions as the primary means of wetland protection (Blumm and Zaleha 1989). The regulation of wetlands fall under section 404 of the U.S. Clean Water Act (CWA)(Clean Water Act 1972).

Determining which wetlands qualify for regulation under the CWA is contentious. Isolated wetlands by definition lack surficial connection to other water bodies (Cowardin et al. 1979, Tiner 2003). Since they lack a permanent, surface-water connection to navigable waterways and do not affect interstate commerce (Zedler and Kercher 2005), GIWs are not subject to federal jurisdiction (Gibbons et al. 2006). However, despite their physical isolation, these GIWs are biologically and functionally connected to other wetlands (Tiner 2003). Two U.S. Supreme Court cases, *Solid Waste Agency of Northern Cook County (SWANCC) v United States*, *Rapanos v United States*, and *Carabell v United States* redefined how we approach the regulation of wetlands in the U.S. (Leibowitz et al. 2008), but small GIWs (less than 4 ha) are excluded from federal protection and protections but may be regulated by state and local governments (Semlitsch and Bodie 1998, Leibowitz and Nadeau 2003). GIWs are not regulated in Georgia (Christie and Hausmann 2003).

The protection of wetlands is vital for conservation of diversity. About half of the threatened or endangered fauna in the U.S. depend on wetlands, and about a quarter of threatened or endangered plants are associated with wetlands (Mitsch and Gosselink 2007). Wetlands are hotspots of global biodiversity (Hannson et al. 2005) and GIWs provide amphibians habitat with low predator density (particularly predatory fish) and high primary productivity (Wassersug 1975, Liu et al. 1997, Ryan and Winne 2001). GIWs support complex food webs (Opsahl et al. 2010), and the interactions between organisms within and around GIWs make them vital for nutrient and biomass conversion and transport (Earl et al. 2011, Capps et al. 2014, Capps et al. 2015). GIWs are the principal breeding sites for amphibians within our model ecosystem (Jensen 2008).

Variations in vegetation and hydroperiod of GIWs in the Dougherty Plain contribute to the support of high amphibian diversity. GIWs in the region include marshes, cypress savannas, and cypress-gum swamps (Battle and Golladay 2001). Marshes are characterized by open canopies, with dense, inundated mixed grasses forming the majority of the physical structure within wetlands (Kirkman et al. 2000). Cypress savannas have a grassy understory with an open, intermediate-level canopy of pond cypress (*Taxodium ascendens)*. Cypress-gum swamps, lastly, are characterized by a closed canopy of cypress and swamp tupelo (*Nyssa sylvatica* var. *biflora)* trees with a sparse hardwood understory (Craft and Casey 2000). GIWs embedded within longleaf pine forest exhibit exceptionally high floral and faunal diversity (Guyer and Bailey 1993, Kirkman et al. 1999, Smith et al. 2006).

Landscape Ecology and Landscape Genetics

Landscape ecology focuses on the interactions between spatial patterns and ecological processes to quantitatively address population shifts in a dynamic environment. The ability of individuals to move freely through landscapes determines species occurrence and persistence. Previous studies addressing landscape connectivity focused on structural connectivity and have been largely model-based, from Euclidean patch distance analysis (MacArthur and Wilson 1967) to least-cost modeling (Adriaensen et al. 2003), individual-based models (Cowen et al. 2006), circuit theory (McRae et al. 2008), and patch theory models (Saura and Torne 2009). These models often use “expert opinion” to determine habitat suitability, usually stemming from the inability to quantify habitat suitability or connectivity. Many models use a *landscape resistance* approach, in which landscape surfaces are parameterized to allow researchers to model animal movement. Landscape resistance may be represented using the inverse of habitat suitability for an organism, but this approach may conflate movement and resource use (Zeller et al. 2012)

Landscape genetics, together with the rapid reduction in cost of genomic sequencing, provides a new framework for the examination of landscape connectivity (Spear et al. 2010). Landscape genetics integrates landscape ecology, spatial statistics, and population genetics to address the intersection of spatial distribution and genetic variation, i.e. the effects of landscape structure on gene flow (Manel et al. 2003, Storfer et al. 2010). Researchers have embraced landscape genetics as a way to identify past barriers to gene flow (Landguth et al. 2010) and as a tool for the conservation of imperiled charismatic species (Short Bull et al. 2011, Wasserman et al. 2013). The challenges of landscapes genetics include questions of optimal scale, simulations, assumptions, and analyses (Balkenhol et al. 2009, Cushman and Landguth 2010, Epperson et al. 2010, Segelbacher et al. 2010, Graves et al. 2013). However, the opportunities afforded by this new discipline are vast. Improvements in molecular genetics, alongside more powerful computational ability and statistical tools, have allowed studies to analyze interactions among populations without preliminary delineation of local populations, thus improving accuracy and limiting costs.

The bulk of genomic cost-reduction is due to a paradigm shift in the sequencing of DNA—from Sanger sequencing to next-generation sequencing (NGS). Although Sanger sequencing is the gold standard of sequencing technology, the aggregate results of NGS sequencing are faster and have higher power, as they process millions of reads per run as opposed to 96 (Mardis 2008). The Human Genome Project illustrates the rapid cost-reduction and increased pace of genomic sequencing; the project, which began in 1990, took 13 years and $2.7 billion USD to complete. Accomplishing identical genomic mapping in October 2015 would take days and cost $1,245 (Wetterstrand KA. DNA Sequencing Costs: Data from the NHGRI Genome Sequencing Program (GSP)). New sequencers and techniques make the production of *de novo* sequences possible within the time and labor constraints of normal research grants. I used 3RADseq double-digest protocols for my examination of amphibian population dynamics.

Restriction-site associated DNA sequencing (RADseq) was developed as a way to examine genetic population dynamics (Davey and Blaxter 2011). It is an effective way to assay population structures of many species, as the identification of genetic markers and the determination of populations' genetic relatedness are combined into a single step, unlike similar microsatellite techniques (Lozier 2014). RADseq enables genotyping and SNP (single-nucleotide polymorphism) discovery, allowing the identification and scoring of genetic markers or tags. This methodology generally requires samples from only 6-10 individuals per population to estimate the genetic distance between populations. Double-digest RADseq protocols differ from basic RADseq in two aspects: the DNA digestion process uses two restriction enzymes, which makes the creation of a DNA library much less expensive, and tags allow a much more precise selection of genomic fragments by size (Peterson et al. 2012). Landscape genetics using NGS is ideally suited to test the effects of structural connectivity on functional connectivity (i.e., movement and gene flow) of populations as a regional scale (Holderegger and Wagner 2008). Use of these protocols, therefore, can quantitatively address questions of landscape resistance, which in turn leads to an understanding of gene flow and the application of best practices in conservation biology.

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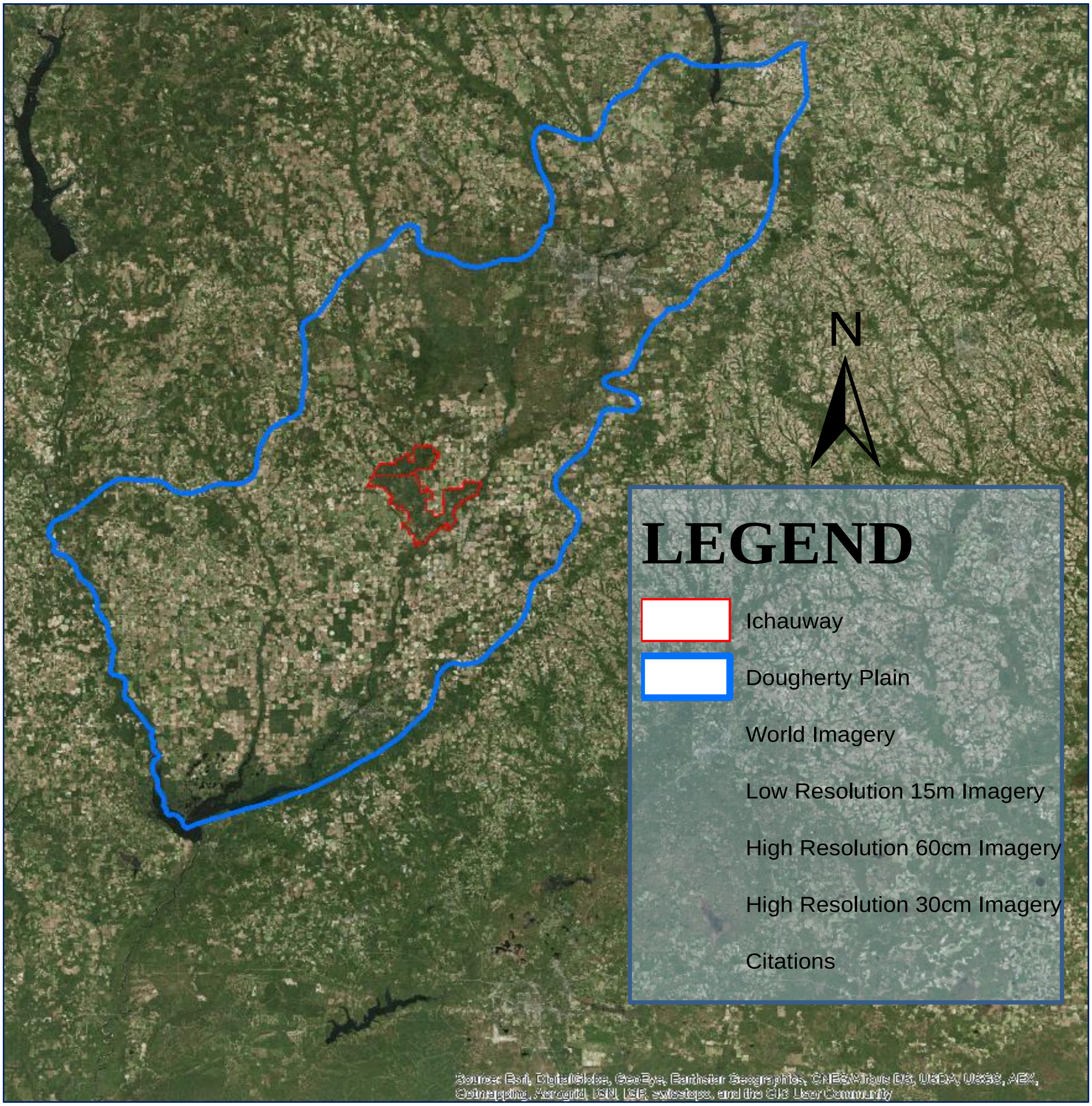

Fig. 1.1. An aerial view of the Dougherty Plain in southwestern Georgia, with Ichauway, the research site of the Joseph W. Jones Ecological Research Center, outlined in red.

Fig. 1.2. An aerial photo of geographically isolated wetlands (GIW) sampled for amphibians in southwestern Georgia in 2015-2016. Clockwise, outsets show a) longleaf pine uplands, b) a GIW surrounded by forested uplands, c) a GIW embedded in agriculture, and d) a center-pivot irrigated field.

CHAPTER 2

CONNECTIVITY THROUGH COMPOSITION: THE EFFECTS OF LANDSCAPE FEATURES ON AMPHIBIAN ABUNDANCE[1]

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[1]McElroy, C.L., L.L. Smith, J. Hepinstall-Cymerman. To be submitted to *Landscape Ecology*.

ABSTRACT

Landscape composition and configuration influence ecological processes, including wildlife population dynamics. Therefore, anthropogenic alterations of landscapes may affect populations. Effects of landscape changes are poorly understood, particularly for organisms with complex life histories (e.g., amphibians). To understand the effects of land-use alteration and landscape features on amphibians, I sampled amphibian species composition and abundance at 33 geographically isolated wetlands (GIWs) in the Dougherty Plain physiographic province of Georgia, USA. GIWs are abundant throughout the Dougherty Plain, despite widespread land-use conversion to irrigated agriculture, which peaked in the 1970's. I measured amphibian species diversity and abundance in GIWs embedded within a land-use gradient from forest to agricultural/urban and used negative binomial generalized linear models to determine the effects of land-use/land-cover on amphibian communities. I captured 21 amphibian species from May 2015 – October 2015 and February 2016 – April 2016. GIWs in landscapes with higher forested land-use (i.e., >45% forest within a 250 m buffer) had higher diversity (2.22 Shannon-Weiner Index) than those within altered landscapes (1.91 Shannon-Weiner Index), but the amphibian communities were fairly similar among GIWs (0.47 Bray-Curtis dissimilarity index). Percent forest cover and predicted wetlands around GIWs best explained total amphibian abundance, although abundance of one species, the southern leopard frog (*Lithobates sphenocephalus*) was not correlated with these variables.

INTRODUCTION

Amphibian species are in decline globally, with an estimated current extinction rate more than 200 times the background rate (McCallum 2007). A comprehensive assessment of the status of amphibian populations in 2004 suggested that 32.5% of amphibian species were threatened (Stuart et al. 2004). This decline of amphibians is largely due to anthropogenic land-use change, although it is difficult to distill and quantify the effects of large-scale habitat change populations (Wiens 1989, Andren 1994, Cushman 2006, Gardner et al. 2007). Habitat alteration, including the conversion of natural areas to anthropogenic land-use, results in habitat loss and fragmentation (Goodwin 2003) and increases pressure on habitat specialists (Benton et al. 2003). When populations are isolated by patches or altered land-use, dispersal and recolonization become increasingly difficult and species face greater risks of local extinction, as their mortality rates are highest when dispersing through unsuitable habitat (Levin and Paine 1974, Hastings 1980, Hanski 1999). An ontogenetic habitat shift and low vagility, characteristic of many amphibians, compound the need for intact landscapes that include breeding and nonbreeding habitat (Semlitsch and Bodie 2003, Cushman 2006). When habitat loss and fragmentation force individuals to disperse further, more frequently, or through impermeable habitat, amphibians may be vulnerable to road mortality, depredation, and desiccation (Cushman 2006). Moreover, dispersal through unsuitable habitat may have negative sublethal effects (i.e. higher expended energy (Rittenhouse et al. 2009)). Thus, the ability of organisms to move across a landscape to reach habitable landscapes is paramount to species persistence (Fahrig and Merriam 1994, Moilanen and Hanski 1998, Ricketts 2001, Briers 2002).

For amphibians that breed in GIWs, local wetland characteristics such as hydroperiod and vegetation type influence amphibian presence and abundance (Snodgrass et al. 2000, Babbitt et al. 2003). At the landscape scale, studies have suggested that forest area and distance between habitat patches affect local amphibian biodiversity (Gibbs 2000, Guerry and Hunter 2002, Trenham et al. 2003, Herrmann et al. 2005). However, studies which investigate species- or assemblage-specific effects of landscape features on amphibians at multiple spatial scales are rare (Cushman 2006, Veysey et al. 2011). The interactions between local and landscape effects on amphibian presence and abundance are also insufficiently studied (Hamer and McDonnell 2008). Moreover, endemic species or habitat specialists may be more affected by landscape conversion than more generalist species (Griffith and Sultan 2012).

To examine the effects of land-use on amphibian presence and abundance, I conducted amphibian surveys in GIWs within the Dougherty Plain of southwestern Georgia, U.S., across a range of land-use composition. The Dougherty Plain, a physiographic province within the Southeastern Coastal Plain, is characterized by karst topography, well-drained soils, and abundant geographically isolated wetlands (Beck and Arden 1983, Martin et al. 2012). This abundance of GIWs contributes to the high overall herpetological diversity of Georgia (Jensen 2008), and the abundance and distribution of GIWs are ideal for studying the functional connectivity of the landscape. *Functional connectivity* refers to the ability of organisms to move among habitat patches in a landscape. This is related to *structural connectivity*, which refers to the spatial arrangement of habitat patches. The landscape was historically covered by the longleaf pine ecosystem, which has suffered an estimated 97% loss across its range (Frost 1993). Large portions of the Dougherty Plain currently support irrigated agriculture, silviculture, and small urban centers (Martin et al. 2012). The conversion to agriculture peaked in the 1970’s, leaving only isolated patches of intact longleaf pine (*Pinus palustris)* forest and potentially fragmenting amphibian populations. The landscape has changed considerably in the past century; natural forests and unirrigated agriculture have declined, while planted pine (largely silviculture) and irrigated agriculture have expanded; overall, the landscape has become patchier and more fragmented (see Martin 2010). In this study, I examined amphibian community composition, abundance, and population genetics (Chapter 3) to determine how land-use and land-cover around geographically isolated wetlands affects connectivity. I used a modeling approach to quantify the effects of landscape features on longleaf pine specialists as a guild (Guyer and Bailey 1993) and on three anuran species with potentially different dispersal capabilities (*Lithobates sphenocephalus*, *Acris gryllus* and *Pseudacris ornata).*

The southern leopard frog (*Lithobates sphenocephalus*) is a large-bodied (50 to 130 mm snout-to-vent length (SVL))(Jensen 2008) habitat generalist with a corresponding comparatively high dispersal ability. The southern cricket frog (*Acris gryllus*) is small-bodied (15 to 33 mm SVL (Jensen 2008)), which suggests they may have a lower dispersal ability than leopard frogs. The ornate chorus frog (*Pseudacris ornata*) is intermediate in size (25 to 40 SVL (Jensen 2008)) and is described as a longleaf pine specialist (Guyer and Bailey 1993), which may translate to restricted movement patterns through altered uplands that surround breeding wetlands. Understanding species- and assemblage-specific responses to landscape features is vital for the creation of scientifically justified management and conservation planning, especially for specialist amphibian species which rely on GIWs and the nearly extirpated longleaf pine forest.

To this end, I examined the amphibian community composition and abundance within GIWs embedded across a gradient of land-uses, from mature longleaf pine forest to irrigated agriculture. I created generalized linear models using negative binomial regression to test for the effects of landscape features on amphibian communities, specifically the abundance three anuran species and a suite of longleaf pine specialists at spatial scales potentially relevant to dispersing or migratory amphibians (100 m – 1000 m buffers around study GIW). I hypothesized that amphibian abundance would increase with the level of surrounding forest and wetland habitat. In contrast, I predicted that amphibian abundance would decrease with increased agricultural land-use and/or increased road density. For the three modeled species, I expected forest to have the greatest effect on ornate chorus frogs, due to their relationship with longleaf pine, and I expected predicted wetlands to have the greatest effect on southern cricket frogs and ornate chorus frogs, due to their smaller body size. I expected the abundance of all species to have a positive relationship with forest cover and the presence of GIWs, albeit to varying degrees.

METHODS

Study Area

I conducted this research within the Dougherty Plain physiographic region in southwestern Georgia within 25 km of and on Ichauway, the 11,600 ha research site of the Joseph W. Jones Ecological Research Center. Ichauway is managed as a bobwhite quail (*Colinus virginianus*) hunting plantation and longleaf pine reserve, through frequent prescribed fires on approximately two-year intervals and small planted food plots. Ichauway includes 7,250 ha of mature (70-95 year old) longleaf pine forests with intact native ground cover, with the remaining land consisting of slash (*Pinus elliottii*) and loblolly (*P. taeda)* pine forests, mixed pine-hardwood forests and lowland hardwood hammocks, and nearly 100 GIWs (Boring 2001). The dominant current land-use of the surrounding Dougherty Plain includes large-scale irrigated agriculture and silviculture operations, as well as private quail-hunting plantations and small urban centers. GIWs are abundant throughout the Dougherty Plain (Martin et al. 2012), although conditions of wetlands in the region vary depending on land-use (Stuber et al. 2016).

Site Selection

I identified study wetlands on Ichauway and the surrounding landscape several existing spatial layers and ArcGIS software (Ver. 10.2, ESRI, Redlands, CA, USA). Wetlands outside of the Ichauway plantation were chosen largely due to prior access permission from landowners. I integrated non-permanent waterbodies from the National Hydrography Database (NHD) (U.S. Geological Survey, accessed through Georgia GIS Clearinghouse, https://data.georgiaspatial.org/index.asp, accessed October 2016) with aerial photos (ArcGIS Basemap, Google Earth) and prior access permissions (Stuber 2013) to determine potential study GIWs. I combined spatial GIW layers with 2011 National Land Cover Data (NLCD) to assess land-use/land-cover surrounding potential study wetlands. Landscape variables included road density (Georgia Department of Transportation, accessed through Georgia GIS Clearinghouse, https://data.georgiaspatial.org/index.asp, accessed October 2016), and proportions of agriculture, urban, and forest land-cover adjacent to wetlands. I selected wetlands that occurred across a gradient of land-use alteration, from reference GIWs (embedded in longleaf pine forest patches) to highly disturbed (embedded in agricultural land-use).

I chose 36 GIWs located on or within 25 km of Ichauway that occurred within complexes, wherein a focal wetland had two or other more GIWs within 350 m of its borders; this configuration was chosen to address questions regarding amphibian population genetics

(Chapter 3). Six wetland complexes (18 GIWs) were within Ichauway, and six (18 GIWs) were within 25 km of Ichauway, including sites on both public and private lands (wildlife management areas, private hunting plantations and row-crop agricultural lands).

Field Methods

I used two methods to detect amphibians within study wetlands: dipnetting for aquatic and larval amphibians, and audio recordings to identify calling adult anurans. I dipnetted amphibians during two field seasons, from May to October 2015 and February to April 2016. Surveys were completed once per month if the wetland held water. Generally, wetlands in the region fill during winter and early spring and dry down in late summer or fall (Battle and Golladay 2002). Each survey consisted of a total of 150 dipnet sweeps per wetland. Sweeps were approximately 1 m in length and I used a heavy-duty D-frame net with a 3 mm mesh size (Memphis Net and Twine, Memphis, TN). Sweeps were distributed across the wetland to sample all microhabitats based on vegetation type and water depth. I identified all amphibians and collected up to 30 larvae of three target species (*L. sphenocephalus, P. ornata* and *A. gryllus*) for use in genetic analyses (Chapter 3). Several larvae were reared in the lab to confirm species identification. After identification, the juvenile specimens were released at the site of capture. All other amphibians were released immediately after capture. The use of animals in this study was approved by the University of Georgia's Animal Care and Use Committee (AUP # A2015 02-012-Y1-A0) and the Georgia Department of Natural Resources Scientific Collection Permit (# 29-WJH-14-156).

I also monitored calling anurans using a froglogger (Songmeter model SM3, Wildlife Acoustics Inc., Maynard, MA) to record from one hour after sunset to sunrise, five minutes at the top of the hour every hour, for three consecutive nights per month, from January – June 2016. A listener identified all calling anurans to species.

Statistical Analyses

Amphibian Community Metrics: Species Richness, Diversity, and Similarity

I estimated amphibian species richness for each wetland using Estimate S software Ver. 9.1.0 (Colwell 2013). I calculated diversity indices for individual wetlands using the Shannon-Weiner Index and classic Chao estimates (Chao et al. 2005) and examined community similarity using the Bray-Curtis abundance-based similarity index (Bray and Curtis 1957) and the Morisita-Horn similarity index (Magurran 1988). Because previous studies have suggested that surrounding forest cover is a predictor of amphibian diversity (Guerry and Hunter 2002, Herrmann et al. 2005, Farmer et al. 2009), I categorized wetlands as "forested" (>45% forest within a 250m buffer) or "altered" (<45% forest within a 250m buffer) in some comparisons. This extent was chosen as it was the best scale for modeling total amphibian abundance, according to my model rankings.

Landscape-Scale Predictors of Amphibian Abundance and Community Composition

To assess relationships among amphibian community composition, amphibian abundance, and surrounding land-use, I quantified land-use/ land-cover composition (described below) in 100 m, 250 m, 500 m and 1000 m buffers around each wetland using ArcGIS Version 10.2 (ArcGIS 2011). I chose these buffer sizes because 100 m is the buffer size used by the Landscape Development Intensity index (LDI), a method often used to remotely assess wetland conditions (Brown and Vivas 2005), while 250m, 500m and 1000m buffers encompass migration and dispersal distances of most amphibians (Berven and Grudzien 1990, Semlitsch and Bodie 2003, Gamble et al. 2007, Greenwald et al. 2009), although some species are capable of longer migrations (Smith and Green 2006).

I used data from the 2011 National Land Cover Database (NLCD) to calculate the proportion of land-use and land-cover types within the buffers described above (Table 2.1). The landscape variables were proportion of forest, agriculture, wetlands, predicted wetlands, and road density within the respective buffer extent, with a local variable of dominant wetland vegetation (primarily forested, classified as swamp, or primarily grassy, classified as marsh). I grouped NLCD classes to reduce the number of variables and allow modeling based on predicted permeability for migrating amphibians, as follows: “Forest” included Deciduous Forest (41), Evergreen Forest (42), and Mixed Forest (43), “Wetlands” included Woody Wetlands (91) and Emergent Herbaceous Wetlands (92), and “Agriculture” included Pasture/ Hay (81), Row Crops (82), and Urban/ Recreational Grasses (85). I included the variable of road density (scaled for intensity using speed limits, under the assumption that higher speed roads would prove a greater impediment to amphibians through increased mortality). Lastly, I included a variable of predicted isolated wetlands (hereafter predicted wetlands), determined through the interaction of hydric soils and topography, using a layer created by Glenn Martin (2010). I used negative binomial regression within the generalized linear modeling framework in Program R (package MASS) to identify important predictor variables for amphibian abundance. I used counts of individuals as a proxy for species abundance. I tested the collinearity of variables using variance inflation factors (VIFs) (Mansfield and Helms 1982) and Pearson correlations; with the regressor variables in my models, the suggested VIF cutoff was 3. Land-use variables were continuous (0-1 for wetlands, forest, agriculture, and road density, prior to arcsine square root transformation

(Ahrens et al. 1990)) and wetland type was categorical (swamp or marsh). I used Akaike's Information Criterion (AICc), which corrects for small sample size (Burnham and Anderson 2002), as an information theoretic approach to identify the model of best fit to my data and to evaluate relative support for competing models. I considered parameters as informative if their 95% confidence interval (CI) did not overlap zero (Burnham and Anderson 2002).

I created five *a priori* models (Table 2.2) to test effects of landscape and wetland-scale variables on the abundance of *A. gryllus, P. ornata,* and *L. sphenocephalus* and a guild of longleaf pine specialists (Table 2.3). Amphibians have been classified as longleaf pine habitat specialists based on either the overlap of their range with that of the historic range of longleaf pine (Guyer and Bailey 1993, Klaus and Noss 2016) or by habitat obligation or sensitivity (Wilson 1995, Schurbon and Fauth 2003, Means et al. 2004) (Table 2.3). If two or more of the five preceding publications classified a species as a longleaf pine specialist, I defined it as such. Models were run for multiple spatial scales (buffer sizes of 100 m, 250 m, 500 m and 1000 m), and the top models (1 – 5, Table 2.2) from each buffer (if applicable) were selected using AIC_C (Burnham and Anderson 2002).

RESULTS

Sampling occurred from May 2015—October 2015 and February 2016 – April 2016; all wetlands were dry from November 2015 to January 2016. I attempted to survey 36 GIWs in 12 wetland complexes monthly. Three wetlands were dry throughout the study; hence, a total of 33 wetlands (13 swamps and 20 marshes in 12 wetland complexes) were included in the analyses.

Amphibian Community Metrics

I detected amphibian larvae in 30 of the 33 study GIWs. Most larval amphibians were identified to species, but I was unable to differentiate larvae of the striped newt *Notophthalmus perstriatus* and eastern newt *Notophthalmus viridescens* and grouped these as one species "NOspp". I was also unable to differentiate tadpoles of *Pseudacris feriarum* and *Pseudacris nigrita* and classified collectively these as "PSspp". I captured a total of 21 amphibian species during dipnetting surveys, encountering 20 amphibian species in wetlands within forested upland landscapes (>45% forest within a 250 m buffer, n=16) and 19 species in wetlands within altered landscapes (<45% forest within a 250 m buffer, n=17) (Table 2.3). Due to small sample sizes, I used Chao parameter protocols to estimate species richness. The Chao1 estimate for GIWs in forested landscapes was 20 (20 – 20.3 95% CI) species and the Chao1 estimate for GIWs in altered landscapes was 22 (18.56 – 24.5 95% CI) species. The Bray-Curtis abundance-based similarity index comparing forested GIWs to altered GIWs was 0.47 (from 0 – 1, with 0 representing no community similarity among wetlands and 1 demonstrating identical communities.) The Shannon-Weiner Diversity index was 2.18 within forested GIWs, 1.93 within altered GIWs, and 2.19 in all GIWs (Table 2.3).

Landscape Predictors of Amphibian Abundance and Community Composition

Of the 33 GIWs sampled, 14 were swamps and 19 were marshes. Land-use parameters varied across levels of alteration on the landscape (Table 2.3). Two parameters (proportion of forest and proportion of agriculture) were correlated (R^2 = 0.52, VIF= 3.23), and therefore agriculture was removed from analyses.

Southern cricket frog abundance was best explained by the global model and an interaction between wetland and predicted wetlands at a buffer size of 1000 m (w_i = 0.996; Table 2.5). The variables of predicted wetland, forest, and the interaction between proportion of wetlands and predicted wetlands were significant (Table 2.5) (p <0.05), and graphical interpretation of this interactive relationship is shown in Fig. 2.1.

The top model explaining ornate chorus frog abundance contained an interaction between forest and predicted wetlands as well as wetland vegetation at the smallest buffer size of 100 m (w_i = 0.911; Table 2.6), as demonstrated in Fig. 2.2.

Southern leopard frog abundance was best explained by the model which included the interaction of forest and predicted wetlands as well as wetland vegetation type at a 250 m buffer size (w_i = 0.550; Table 2.7). Although these models were the most supported, they only contained wetland vegetation type as a significant factor (Table 2.7).

The top model for the prediction of abundance of longleaf pine specialists included an interaction of forest and predicted wetlands as well as wetland vegetation type at a 250 m buffer size (w_i = 0.337; Table 2.8). Forest was a significant predictor variable in the two top models and wetland was a significant variable in the second-best model.

DISCUSSION

Overall species richness across study wetlands was 21, with 20 species occurring in GIWs in forested uplands and 19 species occurring in GIWs in altered uplands. According to Chao estimates, it is likely I detected all or nearly all amphibian species breeding within the GIWs; however, amphibian population may fluctuate widely from year to year (Marsh 2001). The confidence intervals for Chao1 estimates were larger for wetlands within altered uplands.

This larger confidence interval indicates greater variability of wetland abundance, as Chao indices are calculated partially by the incidence of singles and doubles (species with one or two individuals encountered per wetland). This was largely a function of wetlands within altered surroundings sustaining less robust or abundant populations of some sensitive species (namely, singles of mole salamanders, tiger salamanders, pine woods treefrogs, and little grass frogs) (Table 2.3). GIWs surrounded by forested upland had higher diversity (2.22 Shannon-Weiner index) than the GIWs in altered land-use (1.91 Shannon-Weiner index), but the communities were fairly similar (0.47 Bray-Curtis index). Two amphibian species, the gopher frog (*Lithobates capito)* and the southern chorus frog (*Pseudacris nigrita)*, were found exclusively in wetlands within forested uplands, while the eastern narrowmouth toad (*Gastrophryne caroliniensis)* was exclusively found in wetlands within altered uplands. Gopher frogs are associated with gopher tortoises (*Gopherus polyphemus*), whose burrows they use as refugia (Roznik and Johnson 2009). Their exclusion from wetlands, therefore, is unsurprising, as conspecific gopher tortoises will avoid bad habitat (McCoy et al. 2013). In a similar vein, southern chorus frogs have been identified as longleaf pine specialists (Klaus and Noss 2016). Eastern narrowmouth toads breed in a wide variety of wetlands (Dodd and Cade 1998), and often forage at a considerable distance from the nearest pond (Dodd Jr 1996). These generalist tendencies may explain their abundance in wetlands embedded in altered uplands, but do not explain why they were not also encountered in wetlands surrounded by forested uplands; this could have been a result of the timing of sampling, wherein the wetlands in altered uplands had longer hydroperiods due to the effects of irrigation.

These levels of diversity and community similarity among wetlands could be valuable for land managers concerned with maintaining overall amphibian biodiversity. This may be particularly true as the Coastal Plain is the most herpetologically diverse region in Georgia (Jensen 2008) and the southeastern U.S. is a herpetofaunal hotspot (Graham et al. 2010). There are 29 anuran species and 12 salamander species native to the southeastern Coastal Plain that use GIWs as breeding habitat, either exclusively or in addition to other aquatic habitats (Moler and Franz 1987, Conant and Collins 1998), with at least 35 of these native to historic longleaf pine forests (Guyer and Bailey 1993, Means et al. 2004). Moreover, several amphibian species in the Southeastern Coastal Plain are designated as endangered, threatened, or rare by the Georgia Department of Natural Resources, including the flatwoods salamander (*Ambystoma bishopi* or *A. cingulatum)*, the gopher frog, and the striped newt (*Notophthalmus perstriatus*). Even wetlands within extremely altered uplands supported many amphibian species, although abundances among wetlands may differ.

However, scientists and managers may have goals aside from the conservation of overall biodiversity. Although biodiversity is a common and encompassing goal, it may be difficult to define or overvalue species quantity or "quality" over rarity (Gotelli and Colwell 2001, Wätzold et al. 2006). Therefore, studies which quantify and account for variations in species composition in response to land-use alteration are important to the preservation and sustained vitality of amphibians. I found that GIWs embedded in forested uplands were more likely to support higher abundances of rare and more environmentally sensitive species, including some defined as longleaf pine specialists.

Few studies have examined species- and assemblage-specific multi-scaled effects of land-use (Hamer and McDonnell 2008). Amphibians are often managed as a group as opposed to individual species with diverse natural histories and habitat requirements (Barrett and Guyer 2008). I compared landscape effects among species with divergent body sizes (*Acris gryllus,* *Lithobates sphenocephalus*, and *Pseudacris ornata*) and longleaf pine specialists. My results demonstrated notable disparity among amphibian responses to land-use/land-cover. I used amphibian (individual) counts as a proxy for abundance; count data has been shown to be roughly proportional to population size (Slade and Blair 2000), is commonly used as an index of abundance in studies of terrestrial and aquatic amphibians (Dodd and Dorazio 2004), and can be appropriate for species that forage or breed in specific habitat (Sharples et al. 2009) (i.e. amphibian larvae in isolated wetlands). Count data do have limitations; amphibian abundance varies temporally, as it is highly dependent on variable environmental conditions such as precipitation and hydroperiod (Pechmann et al. 1989). Amphibian abundance and detection probabilities vary widely from year-to-year (Dodd and Dorazio 2004). Count data, therefore, is unsuitable for monitoring amphibian population trends, especially in studies which span multiple years. As I was using these counts as a variable in single-species or guild models which examined potential landscape effects on amphibians as opposed to determining long-term population viability, I found count data to be an acceptable proxy for amphibian abundance.

Model results for southern leopard frogs were difficult to interpret. Only the vegetation within wetlands had any significant effects on abundance, with marshes supporting slightly greater southern leopard frog abundance than swamps. The southern leopard frog was the only species whose abundance was positively associated with roads, although this association was not statistically significant. While roads generally have a negative effect on amphibians due to vehicle-caused mortality or the fragmentation of landscape (Fahrig et al. 1995, Mazerolle 2004, Gibbs and Shriver 2005, Glista et al. 2008, McKee et al. 2011), some organisms use roads as corridors, including cane toads (*Bufo marinus*) (Seabrook and Dettmann 1996, Lugo and Gucinski 2000, Brown et al. 2006). Leopard frogs are large-bodied, and could theoretically use roads to aid in dispersal and migration across the landscape. The leopard frog is considered a habitat generalist because it has a broad geographic range (much of the Eastern U.S.) and it occurs in a wide variety of habitats (Gregoire and Gunzburger 2008), which would seem to be supported by our models.

Southern cricket frogs, ornate chorus frogs, and the longleaf pine specialists were influenced strongly by forest cover in the surrounding landscape. Forest cover is often the primary factor associated with amphibian presence and biodiversity (Guerry and Hunter 2002, Werner et al. 2007, Farmer et al. 2009). Previous literature has linked ornate chorus frogs to intact, forested landscapes (Todd et al. 2009), and longleaf pine specialists and longleaf residents depend on open-canopy pine forests (Guyer and Bailey 1993) due to evolutionary associations with the pyrogenic longleaf ecosystem (Steen et al. 2010).

Predicted wetlands were positively correlated with the abundance of southern cricket frog and ornate chorus frog populations. The layer I adopted to classify predicted wetlands (Martin 2010) was largely dependent on the occurrence of hydric soils (Martin et al. 2012), which have been linked to amphibian presence (Gibbons 2003). Amphibians are often linked to measures of soil moisture, though to varying degrees (Thorson and Svihla 1943, Wyman 1988, Conant and Collins 1998). Both ornate chorus frogs and southern cricket frogs are small-bodied and may be more susceptible to desiccation than other amphibian species. Because amphibians lose water readily through their permeable skin (Pounds and Crump 1994) and do not drink, their primary source of water is the moisture in soils (Tracy 1976), and the proximity to moist hydric soil could be important to their survival.

Contrary to my *a priori* hypothesis, GIWs with a high proportion of wetland land-cover in the surrounding landscape were negatively correlated with amphibian abundance. Initially this result appears counterintuitive, as wetland density is thought to support high amphibian diversity (Semlitsch and Bodie 1998). But, perhaps the most isolated support greater abundance and diversity because they are the only available wetlands, whereas aggregated wetlands might collectively support greater diversity (Kirkman et al. 2012).

There is a wealth of literature on the negative effects of habitat loss and fragmentation on amphibian populations (Fahrig 1997, Semlitsch and Bodie 1998, Benton et al. 2003, Fahrig 2003, Revilla et al. 2004, Pimenta et al. 2005, Becker et al. 2007). Despite global declines of amphibians and other populations, largely due to habitat loss, it is not feasible to halt the expansion of agriculture, silviculture, and urbanization. Human populations continue to grow, and these increases lead to expanded requirements for resources and living space (Meyer and Turner 1992, Cincotta et al. 2000, McKee et al. 2004, Wittemyer et al. 2008, Martin et al. 2012). Therefore, habitat conversion is difficult to reverse and unlikely to slow. Instead, land managers and conservationists must be equipped with the knowledge necessary to preserve amphibians (especially endemic or specialist species) without impeding anthropogenic development. My study demonstrated that some species of amphibians were surprisingly resilient to changes within the landscape, but if researchers can discover which landscape features are vital to the retention of functional connectivity on a landscape, they can make scientifically supported, fully informed decisions as to which tracts of land to purchase, preserve, and protect. While GIWs in more natural uplands support more rare species and higher biodiversity, including gopher frogs and southern chorus frogs, protection of complexes of wetlands— regardless of surroundings—is the best solution to curtail species loss and maintain the entire species assemblage in the region. Those wetlands embedded in forested areas, especially within relict longleaf pine or forested habitats, should be afforded particular protection.

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Table 2.1. Landscape-scale variables included in generalized linear models to explain amphibian abundance in geographically isolated wetlands in southwestern Georgia in 2015-2016

Variable	Description and Source	Predicted Effect on Amphibian Abundance
Forest	Proportion of forest within buffer. Forest includes “deciduous forest”, “evergreen forest” and “mixed forest” layers (41, 42, 43) in 2011 NLCD.	↑
Wetland	Proportion of isolated wetlands within buffer. Derived from “woody wetlands” and “emergent herbaceous wetlands” layers (91, 92) in 2011 NLCD, combined with discovered wetlands from ground-truthing and Google Earth.	↑
Agriculture	Proportion of agriculture within buffer. Includes “pasture/ hay”, “row crops”, and “urban recreational grasses” layers (81, 82, 85) in 2011 NLCD.	↓
Road	Proportion of roads within buffer. Derived from road layers in 2011 NLCD, a specialist road map from Ichauway, and mph limits from GA DOT. Scaled so higher-mph roads (highways, etc) count more than sand firebreaks.	↓
Pred.wet	Proportion of ground which has the potential to be a wetland. Derived from a layer created by Glenn Martin for the Dougherty Plain (Martin et al. 2012).	↑
Offset	Variable used to transform models for individual species into a comparable form	

Table 2.2. *A priori* models tested at each buffer size (100 m, 250 m, 500 m, and 1 km) for abundance of three amphibian species geographically isolated wetlands in southwestern Georgia in 2015-2016.

Model #	
1	wetland + forest + wetland vegetation + road + predwet + offset
2	predwet * forest + wetland vegetation + wetland + road + offset
3	predwet * forest + wetland vegetation + road + offset
4	forest * predwet + wetland vegetation + offset
5	wetland * predwet + road +forest + wetland vegetation + offset

Table 2.3. Amphibian species surveyed within GIWs, overall and between wetlands embedded in forested uplands (>45% forest within a 250 m buffer) and wetlands embedded in altered uplands (<45% forest within a 250 m buffer) in southwestern Georgia in 2015-2016. X designates an encountered species, and an asterisk designates a singleton.

Species Encountered (Overall)	GIWs in Forested Uplands	GIWs in Altered Uplands
Southern cricket frog (*Acris gryllus)*	X	X
Mole salamander (*Ambystoma talpoideum)*	X	X*
Tiger salamander (*Ambystoma tigrinum)*	X	X*
Southern toad (*Anaxyrus terrestris)*	X	X
Dwarf salamander (*Eurycea quadridigitata)*	X	X
Eastern narrowmouth toad (*Gastrophyrne caroliniensis)*		X
Cope's gray treefrog (*Hyla chrysoscelis)*	X	X
Green treefrog (*Hyla cinerea)*	X	X
Pine woods treefrog (*Hyla femoralis)*	X	X*
Barking treefrog *(Hyla gratiosa)*	X	X
Squirrel treefrog (*Hyla squirella)*	X	X
Gopher frog (*Lithobates capito)*	X	
Bullfrog (*Lithobates catesbeianus)*	X	X
Southern leopard frog (*Lithobates sphenocephalus)*	X	X
NoSPP (*Notophthalmus perstriatus* and *N. viridescens)*	X	X
Southern chorus frog (*Pseudacris nigrita)*	X	
Little grass frog (*Pseudacris ocularis)*	X	X*
Ornate chorus frog (*Pseudacris ornata)*	X	X
PsSPP (*Pseudacris nigrita* and *P. feriarum)*	X	X

Table 2.4. Amphibian species richness and biodiversity metrics, overall and between wetlands embedded in forested uplands (>45% forest within a 250 m buffer) and wetlands embedded in altered uplands (<45% forest within a 250 m buffer) in southwestern Georgia in 2015-2016.

Community Metrics					
	Species	Chao 1 (CI)	Shannon-Weiner	Bray-Curtis	Morsita-Horn
Altered	19 (13.4 – 20.6)	19 (17.2 – 39.1)	1.93	0.47	0.53
Forested	20 (17.4 – 22.6)	20 (20 - 20.3)	2.18		
All GIWs	21 (20 – 22)	21 (21 – 21.25)	2.19		

Table 2.5. Amphibian species characterized as longleaf pine (*Pinus palustris*) specialists (Guyer and Bailey 1993, Klaus and Noss 2016, Means et al. 2004, Schurbon and Fauth 2003, Wilson 1995).

Scientific Name	Common Name
Ambystoma talpoideum	Mole salamander
Ambystoma tigrinum	Tiger salamander
Hyla femoralis	Pine woods treefrog
Hyla gratiosa	Barking treefrog
Hyla squirrella	Squirrel treefrog
Lithobates capito	Gopher frog
Pseudacris nigrita	Southern chorus frog
Pseudacris ocularis	Little grass frog
PSeudacris ornata	Ornate chorus frog

Table 2.6. Models explaining abundance of the southern cricket frog (*Acris gryllus*) within geographically isolated wetlands in southwestern Georgia in 2015-2016.

	Acris gryllus abundance		
	250 m	500 m	1000 m[1]
Constant	-3.03^{*} (-6.31, 0.24)	-6.08^{***} (-9.93, -2.24)	-9.16^{***} (-14.18, -4.13)
wetland		4.82 (-3.21, 12.86)	8.27 (-5.64, 22.18)
forest	1.02 (-3.62, 5.67)	2.61^{*} (-0.28, 5.49)	4.45^{***} (1.92, 6.97)
predwet	3.09 (-5.56, 11.75)	10.99^{***} (4.65, 17.32)	19.61^{***} (7.93, 31.28)
road		-0.25 (-3.22, 2.73)	1.09 (-2.50, 4.67)
Veg_marsh	0.88 (-0.76, 2.52)	0.78 (-0.59, 2.15)	1.09^{*} (-0.15, 2.33)
forest:predwet	0.82 (-12.74, 14.37)		
wetland:predwet		-17.63^{**} (-33.87, -1.38)	-44.71^{**} (-80.41, -9.02)
Observations	33	33	33
Log Likelihood	-83.37	-78.96	-74.88
Theta	0.23^{***} (0.07)	0.35^{***} (0.12)	0.53^{***} (0.20)
K	6	8	8
W_i	0.002	0.002	0.997
AIC_C	176.74	171.93	163.76
Note:			$^{*}p<0.1$; $^{**}p<0.05$; $^{***}p<0.01$

[1]: Best model for southern cricket frog abundance (W_i= 0.997).

Table 2.7. Models explaining abundance of ornate chorus frogs (*Pseudacris ornata*) in geographically isolated wetlands in southwestern Georgia in 2015-2016.

	Pseudacris ornata abundance	
	100 m[1]	500 m
Constant	0.56 (-1.74, 2.87)	-3.74 (-7.71, 0.22)
forest	-1.66 (-5.13, 1.82)	4.56 (-0.07, 9.20)
predwet	-8.97^{***} (-13.40, -4.55)	-6.14 (-16.66, 4.38)
Veg_marsh	1.16 (-0.32, 2.63)	0.25 (-1.11, 1.61)
forest:predwet	15.04^{**} (7.25, 22.83)	
road		3.26 (0.50, 6.03)
predwet:forest		8.01 (-5.61, 21.63)
Observations	33	33
Log Likelihood	-90.97	-92.29
Theta	0.34^{*} (0.11)	0.32^{*} (0.10)
K	6	7
W_i	0.911	0.089
AIC_C	191.94	196.58
Note:		*p<0.01; **p<0.001; ***p<1e-04

[1]: Best model for southern cricket frog abundance (W_i= 0.911).

Table 2.8. Models explaining abundance of the southern leopard frog (*Lithobates sphenocephalus*) in geographically isolated wetlands in southwestern Georgia in 2015-2016.

	Lithobates sphenocephalus abundance			
	100 m	250 m[1]	500 m	1000 m
Constant	0.23 (-1.66, 2.11)	0.56 (-1.61, 2.74)	-0.25 (-3.01, 2.51)	-0.61 (-3.69, 2.48)
forest	0.59 (-2.34, 3.51)	0.16 (-2.99, 3.32)	1.19 (-2.09, 4.48)	1.74 (-2.37, 5.84)
predwet	-1.73 (-4.78, 1.32)	-3.54 (-9.53, 2.44)	-0.92 (-8.46, 6.63)	2.09 (-7.34, 11.52)
Veg_marsh	1.20^{**} (0.01, 2.38)	0.99^{*} (-0.16, 2.14)	0.66 (-0.47, 1.78)	0.71 (-0.46, 1.88)
forest:predwet	2.39 (-3.67, 8.45)	4.69 (-4.74, 14.13)	2.41 (-7.66, 12.47)	-1.61 (-14.82, 11.60)
Observations	33	33	33	33
Log Likelihood	-130.70	-130.52	-131.26	-132.29
Theta	0.44^{***} (0.11)	0.44^{***} (0.11)	0.42^{***} (0.11)	0.39^{***} (0.10)
K	6	6	6	6
W_i	0.094	0.550	0.262	0.094
Akaike Inf. Crit.	271.41	271.04	272.52	274.57
Note:				$^{*}p<0.1$; $^{**}p<0.05$; $^{***}p<0.01$

[1]: Best model for southern leopard frog abundance (W_i= 0.550).

Table 2.9. Models explaining abundance of amphibians characterized as longleaf pine (*Pinus palustris*) specialists in geographically isolated wetlands in southwestern Georgia in 2015-2016.

	Longleaf Pine Specialists Abundance			
	100 m	250 m[1]	500 m	1000 m
Constant	1.76* (-0.14, 3.66)	1.18 (-1.03, 3.39)	2.09* (-0.33, 4.51)	0.65 (-2.41, 3.71)
wetland			-4.39** (-8.47, -0.31)	
forest	1.62 (-1.32, 4.57)	3.47** (0.27, 6.68)	2.86*** (0.69, 5.04)	3.74* (-0.33, 7.81)
predwet	0.88 (-2.17, 3.93)	3.13 (-2.91, 9.17)	1.27 (-1.21, 3.75)	5.16 (-4.20, 14.52)
Veg_marsh	0.66 (-0.52, 1.85)	0.51 (-0.64, 1.65)	0.66 (-0.42, 1.73)	0.58 (-0.58, 1.74)
forest:predwet	-0.23 (-6.30, 5.84)	-5.22 (-14.74, 4.29)		-6.83 (-19.93, 6.28)
road			-0.47 (-2.82, 1.87)	
Observations	33	33	33	33
Log Likelihood	-149.99	-149.80	-148.93	-151.16
Theta	0.43*** (0.11)	0.43*** (0.11)	0.46*** (0.12)	0.40*** (0.10)
K	6	6	7	6
W_i	0.278	0.337	0.298	0.086
Akaike Inf. Crit.	309.99	309.61	309.85	312.33

[1]: Best model for longleaf pine specialists' abundance (W_i= 0.337).

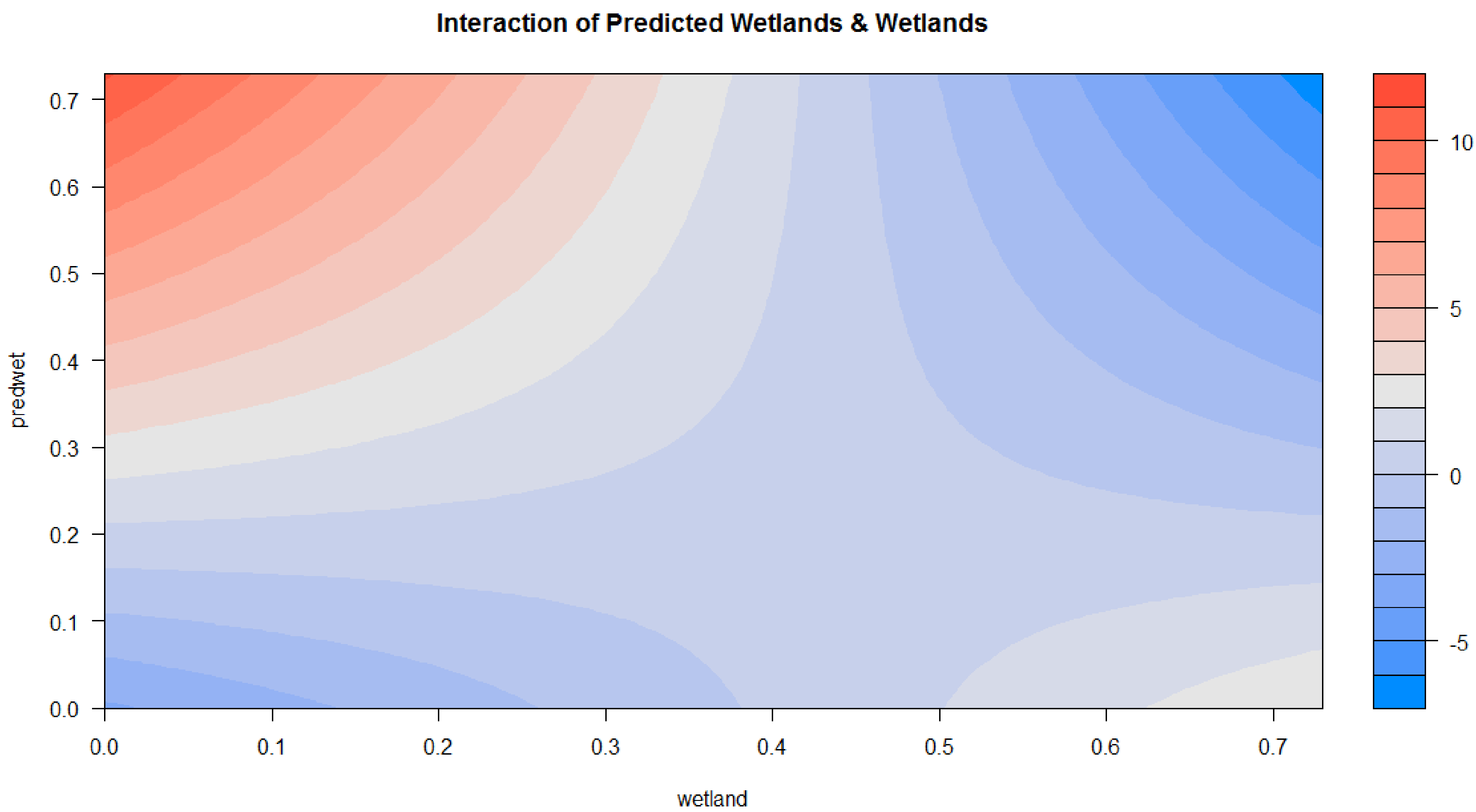

Fig. 2.1. Depiction of the interactions between the proportion of predicted wetlands and wetlands on southern cricket frog (*Acris gryllus*) abundance in geographically isolated wetlands in southwestern Georgia in 2015-2016. The legend shows the scale of land-use effects on abundance of cricket frogs, with red showing the highest abundances and blue showing the most negative effects.

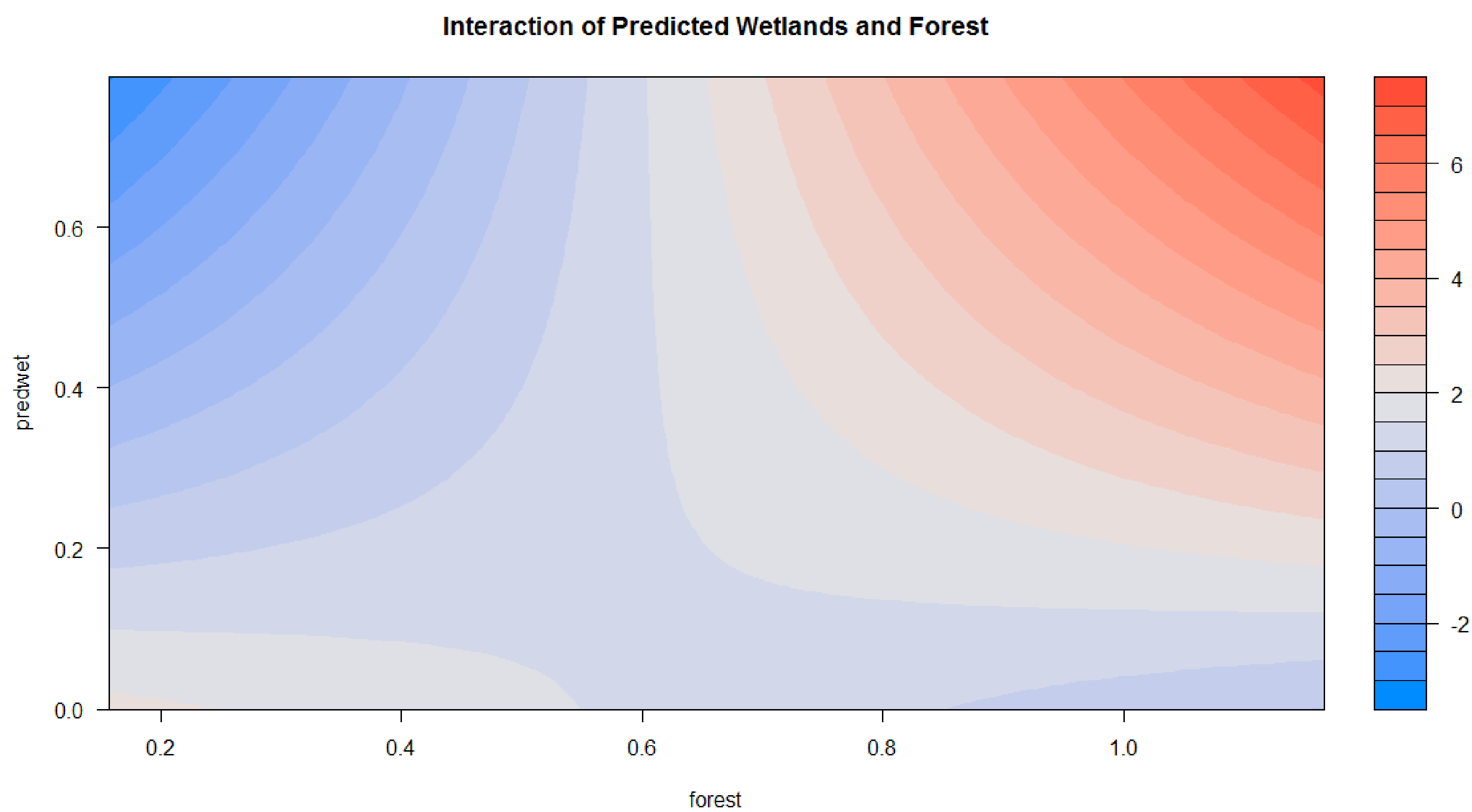

Fig. 2.2. Depiction of the interactions between the proportion of predicted wetlands and wetlands on ornate chorus frogs (*Pseudacris ornata*) abundance in geographically isolated wetlands in southwestern Georgia in 2015-2016. The legend shows the scale of land-use effects on abundance of cricket frogs, with red showing the highest abundances and blue showing the most negative effects.

CHAPTER 3

LEAPS AND BOUNDS: UNDERSTANDING LANDSCAPE CONNECTIVITY THROUGH AMPHIBIAN LANDSCAPE GENETICS

[1]McElroy, C.L., J. Hepinstall-Cymerman, T.C. Glenn, L.L. Smith. To be submitted to *Landscape Ecology*.

ABSTRACT

Amphibians in landscapes with geographically isolated wetlands (GIWs) occur in wetland complexes embedded within an upland matrix that provides habitat for each stage of amphibian ontogeny (i.e. wetlands for breeding and forest for cover and forage). The composition, configuration, and corridors of the landscape influence population dynamics of organisms. I used the genetic differentiation of two amphibian species (southern cricket frogs, *Acris gryllus*, and southern leopard frogs, *Lithobates sphenocephalus)* to examine patterns of connectivity and past gene flow on the Dougherty Plain in southwestern Georgia. I found that Euclidean distance and landscape resistance were correlated with genetic differentiation for populations of southern cricket frogs, but not for the larger, more vagile southern leopard frogs. Models suggested that predicted wetlands around GIWs best explained patterns of connectivity for southern cricket frogs. In addition, models which examined corridors suggested that gene flow among populations of southern leopard frogs in GIWs was facilitated by agricultural or wetland land-use.

INTRODUCTION

Interactions between species and environments are the foundation of ecology. The composition, configuration, and connectivity of landscapes influence dynamic ecological processes which affect the viability of populations and ecosystems (Tischendorf and Fahrig 2000, Bélisle 2005), including the movement of organisms, the spread of diseases, and the dispersal of seeds. Individuals must be able to move through landscapes to discover territories, resources, and reproductive opportunities. Habitat loss and fragmentation can hinder these processes by isolating habitat patches (Smith et al. 2009, Haddad et al. 2015).

The principal cause of habitat loss and fragmentation is anthropogenic habitat conversion; intensive agriculture, increased industrial applications, and growing urban centers have led to extensive deforestation and habitat loss worldwide (Vitousek et al. 1997, Foley et al. 2005). The patterns of land conversion often result in increased extent of edge habitat and decreased interior habitat (Yahner 1988, Fagan et al. 1999), which favors generalist and invasive species over species with more specific niche requirements (Carfagno and Weatherhead 2006). Habitat loss and fragmentation also increase the risk of local extinctions (Harrison 1991, Newmark 1991, Fahrig and Merriam 1994). Habitat loss and fragmentation can lead to increased road mortality (Mazerolle 2004), depredation (Stephens et al. 2004), desiccation (Cushman 2006), and increased energetic costs of migration (Rittenhouse et al. 2009), as well as declines in fitness due to low allelic diversity (Westemeier et al. 1998, Reed and Frankham 2003, Araki et al. 2007).

Allelic diversity refers to number and relative frequency of alleles per locus within a population (Gillespie 2010). The diversity of alleles can be used to examine genetic lineages and subpopulation dynamics (Nei 1987). Anthropogenic habitat loss or degradation can restrict genetic diversity and negatively affect the prospects of species conservation (Mather et al. 2015). Low genetic diversity may be a result of founder effects (Mayr 1942), genetic bottlenecks (Nei et al. 1975), or populations with few remaining individuals (Lande 1988). Although some species appear to be healthy despite their low genetic diversity (Milot et al. 2007), they may be more vulnerable to crises, such as disease or parasite epidemics, than species with robust allelic diversity (Tanksley and McCouch 1997).

To understand how landscape patterns affect population dynamics, we must quantify the effects of landscape permeability and connectivity. Geographically isolated wetlands (GIWs) and the amphibian populations associated with these wetlands can function as ideal model systems for addressing these questions. Past studies addressing questions about amphibian movements to and from breeding sites involved capture-mark-recapture using drift fences (Gibbs 1998) or radio telemetry (Fancy et al. 1988, Hulbert and French 2001), but these techniques are labor-intensive and may be unsuitable for small vertebrates. Alternatively, movement has been examined by modeling "resistance," or the willingness of an organism to cross through different land-covers or land-uses, and is used to predict the likelihood of dispersal through landscapes (Cushman et al. 2010, Zeller et al. 2012). Models of landscape resistance may be created, tested, or improved by incorporating information from population genetics, an application commonly called landscape genetics (Storfer et al. 2007, Holderegger and Wagner 2008).

The field of landscape genetics addresses the interactions among populations and landscape features on a fine spatial and temporal scale (Manel et al. 2003). The ability of organisms to move across landscapes shapes genetic flow by influencing reproductive opportunities (Slatkin 1985, 1987, Bohonak 1999), and the examination of gene flow between populations, can be used to infer past movements (Levin and Kerster 1974). Quantifying the gene flow among populations could allow the identification of landscape features which serve as barriers or catalysts to gene flow and movement (Hitchings and Beebee 1997). Until recently, the time, labor, and cost associated with genetic techniques have limited the amount of genetic information available for landscape-scale studies; however, the advent of massively parallel sequencing techniques has allowed increasing use of spatially explicit genetic variation (Manel et al. 2003, Storfer et al. 2010). Next-generation sequencing (NGS) techniques allow resequencing of full or repeatable fractions of genomes within the time and budget constraints of a typical research grant.

To examine the effects of habitat loss and fragmentation on landscape connectivity and gene flow, I focused on amphibians in GIWs within a portion of the Dougherty Plain. The Dougherty Plain is a physiographic region in southwestern Georgia characterized by karst topography that has been subjected to widespread anthropogenic land-use conversion (Martin 2010, Stuber 2013). There are abundant GIWs (Tiner 2002) on the Dougherty Plain which serve as breeding habitat for a diverse suite of amphibians (Gibbons 2003). Amphibians have limited vagility relative to many other vertebrates and must cross uplands to disperse or migrate to new breeding ponds. Amphibians have high philopatry to their natal ponds as adults (Semlitsch 2008), but GIWs by definition have a variable hydroperiod (Martin et al. 2012), generally filling in the late winter from precipitation and drying in the late summer or fall (Battle and Golladay 2002). This seasonal cycle means that amphibians must develop strategies to survive the periodic drydowns; some amphibians may aestivate during the dry season (Gehlbach et al. 1973), but most disperse in search of favorable non-breeding habitat (Rothermel and Semlitsch 2002, Cushman 2006, Semlitsch 2008), either in search of more permanent waterbodies or adjacent forested upland territories (Semlitsch and Bodie 2003, Homan et al. 2004).

Movement as a response to seasonal drydowns leads to rapid local extinctions and recolonizations within a dynamic regional population (Skelly et al. 1999). These shifts, along with the low relative vagility of amphibians, may highlight the effects of land-use on amphibian movement capability. I considered two anuran species, the southern leopard frog (*Lithobates sphenocephalus)* and the southern cricket frog *(Acris gryllus*) in this study of landscape connectivity. I chose these species because of their abundance in GIWs in this landscape and because their dispersal abilities should be divergent due to the disparity in their body size (Mazerolle 2001). I used the fixation index (F_{ST}), a measure of genetic differentiation among breeding populations (Ahrens et al. 1990), to examine assumptions of amphibian dispersal and landscape connectivity. F_{ST} is a measure of population differentiation which ranges from 0, a completely panmictic population, to 1, complete genetic differentiation of populations (Weir and Cockerham 1984). F_{ST} has been criticized for its dependence on the initial calculation of within-population diversity, but is the most widespread and generally accepted measure of genetic differentiation, as it performs better than R_{ST} (genetic differentiation as estimated from microsatellite data) when limited numbers of individuals and/or loci are genotyped (Balloux and Goudet 2002).

To this end, I obtained F_{ST} values for the pairwise interactions between GIWs and GIW complexes, both for southern leopard frog and southern cricket frog populations. I used these F_{ST} values to examine differences in local and regional populations, test different least-cost path models with resistance values derived from generalized linear models that explained variation in amphibian abundance (see Chapter 2), and finally to create a model which explained the effects of landscape features on observed F_{ST} values. I hypothesized that local populations would demonstrate greater genetic differentiation between GIWs with greater cost distances. I also hypothesized that corridors (in this study, 180 m-wide corridors between GIWs) with high proportions of unsuitable habitat classes (i.e. agriculture, urban) would have negative effects on gene flow and result in more genetic differentiation than those largely characterized by suitable habitats (i.e. forest, wetlands).

METHODS

Study Area

I conducted this research within the Dougherty Plain in southwestern Georgia; all study wetlands were on or within 25 km of Ichauway, the 11,600 ha research site of the Joseph W. Jones Ecological Research Center (Fig 3.1). Ichauway has approximately 7000 ha of longleaf pine forest and more than 90 embedded GIWs. Upland on the property are managed through frequent prescribed fires. This management strategy has preserved the site for the imperiled longleaf pine (*Pinus palustris)* ecosystem. While Ichauway supports pine forests, hardwood hammocks, and small food plots to support the local quail (*Colinus virginianus*) populations (Boring 2001), the surrounding Dougherty Plain has largely been converted to irrigated row-crop agriculture, silviculture, private hunting plantations, and small urban centers (Martin et al. 2012). Despite the variation in land-use and habitat alteration, GIWs are abundant on the landscape, although wetland conditions may vary depending on surrounding land-use (Stuber 2013).

Site Selection

I identified study wetlands on Ichauway using several existing ArcGIS spatial layers (Ver. 10.2, ESRI, Redlands, CA, USA). To identify potential study wetlands I integrated spatial data for non-permanent waterbodies from the National Hydrography Database (NHD) (U.S. Geological Survey, accessed through Georgia GIS Clearinghouse, https://data.georgiaspatial.org/index.asp, accessed October 2016) with aerial photos (ArcGIS Basemap, Google Earth), and regional maps of GIWs developed in previous studies (Stuber 2013). I selected study wetlands that occurred in "complexes" with a core study wetland with two wetlands within 350 m of its border to maximize chances of amphibian movements within wetland complexes. Six wetland complexes were located within Ichauway, and six were within 25 km of Ichauway, including sites on both public and private lands where we had permission to sample (wildlife management areas, private hunting plantations and row-crop agricultural lands) (n=36). I combined spatial data for GIWs with National Land Cover Data (NLCD 2011) to quantify land-use/land-cover surrounding study wetlands.

Focal Species

I chose two locally common species for this study: the southern leopard frog and the southern cricket frog. The southern leopard frog is a relatively large anuran with a snout-vent length (SVL) of 50-130 mm (Conant and Collins 1998, Jensen 2008). Adults have powerful leaping abilities and high vagility in comparison to other anurans (Carr and Fahrig 2001, Graeter et al. 2008, McKee 2012). Movement studies of *Lithobates pipiens*, the northern leopard frog, have concluded that they can disperse as far as 8 km (Seburn et al. 1997, Lehtinen and Galatowitsch 2001, Smith and Green 2006). The relatively low surface-area to volume ratio in leopard frogs may make them less susceptible than smaller anurans to environmental stressors (Lindstedt and Boyce 1985). Southern leopard frogs have a widespread distribution throughout the eastern United States (Gregoire and Gunzburger 2008) and breed in a variety of wetland habitats, including GIWs in proximity to agricultural lands (Alix et al. 2014).

The southern cricket frog is a small anuran (SVL of 15-33 mm (Jensen 2008)). Southern cricket frogs have a powerful leap in proportion to their body size (Blem et al. 1978). Despite their jumping ability, southern cricket frogs are likely to have lower vagility than leopard frogs, due to their larger surface-area to volume ratio and potentially greater evaporative water loss (Tracy 1976) and higher relative expended energy (Rittenhouse et al. 2009). Accordingly, cricket frogs are more likely to remain at wetland margins during the non-breeding season, although they have been observed in terrestrial habitats (Jensen 2008).

Field Sampling

I collected larval and adult anurans for genetic analyses from April 2015—October 2015 and February 2016 – April 2016 using several different methods. First, I used a D-frame dip net (3 mm mesh size, Memphis Net and Twine, Memphis, TN) to collect larvae of the two target species, southern leopard frogs and southern cricket frogs. I collected up to 30 individual larvae of each species per GIW. I also collected up to five adult anurans, using drift fences consisting of 1 m tall silt fencing (Everbilt™) cut into 10 m lengths, placed approximately parallel to and within 10 m of the wetland edge. Pitfall traps consisting of 19 L buckets were placed flush with the ground at the ends of each section of fence. Holes were drilled in the bottom of buckets for drainage and a dampened sponge was used to prevent desiccation of amphibians. Traps were checked once a day for at least four days; when not in use, bucket lids were used to prevent captures. Lastly, I also collected calling adults of the two species at night to obtain the total of five adult individuals of each species from each pond. Adults were collected in addition to larvae to minimize the chance of sampling full siblings. . All specimens collected were stored temporarily in Ziploc™ bags and brought to the lab for processing processed as described below.

Larval and adult anurans were humanely euthanized with Tricaine methane sulfonate (MS-222) (Baumans et al. 1997). I used a buffered solution of 10g/L of MS-222 for adult specimens and 5 g/L for larval specimens (Leary et al. 2013). Specimens were preserved in 70% ethanol and stored in a climate-controlled room until they were processed to isolate and purify DNA. The use of animals in this study was approved by the University of Georgia's Animal Care and Use Committee (AUP #A2015 02-012-Y1-A0) and the Georgia Department of Natural Resources Scientific Collection Permit (#29-WJH-14-156).

DNA Processing

Amphibian samples were processed in the Glenn lab at the University of Georgia. DNA was extracted from indiscriminant soft tissue samples (muscle and organ tissues) from tadpoles and adult anurans using Qiagen DNeasy Blood & Tissue Kit™ materials and protocols. The extracted DNA was replicated for testing using a polymerase chain reaction (PCR) assay for amphibians. All validated DNA was immersed in a double-enzyme digest recipe, including CutSmart™ buffer, distilled water, adapters, and the restriction enzymes Nhe1, EcoR1, and Xba1 (Glenn et al. 2014). Samples were incubated at 20℃ for 20 minutes, 37℃ for 10 minutes, and 80℃ for 20 minutes to denature any remaining proteins and ligate adaptors. Samples were purified with SpeedBeads™. After the preliminary DNA extraction, purification, and normalization described above, created libraries were sent to the Georgia Genomics Facility at the University of Georgia for sequencing on an Illumina NextSeq™. I used double-digest restriction site-associated DNA markers (ddRADseq) protocols, which enable genotyping through the automated scoring of single-nucleotide polymorphisms (Davey et al. 2011, Andrews et al. 2016).

I used the program STACKS (http://catchenlab.life.illinois.edu/stacks/) to process raw sequence data. STACKS includes tools to analyze relatedness of the sampled populations. I received the fixation index values (F_{ST}) for the pairwise comparisons between populations of southern leopard frogs and southern cricket frogs at individual GIWs. Not all samples were appropriate for further analyses. I first removed individuals with a low number of reads (i.e., <500,000). I removed all loci which were not present in at least six populations, and removed loci from populations when loci were not present at least half the individuals. Lastly, I removed all samples from populations which were characterized by 2 or fewer individual frogs, as they were much likelier to result in unsupported, outlying F_{ST} values.

Data Analysis

I tested a series of models that were developed to explain the effects of landscape features on amphibian abundance (see Chapter 2), and created a generalized linear model to quantify the effects of land-use on genetic differentiation (F_{ST}) in the two species.

Then, to test the models that explained variation in amphibian abundance in GIWs across the landscape, I created resistance layers based on my model results. I took values from the prior generalized linear models to reclassify land-cover classes (NLCD 2011) to create a resistance layer (ArcGIS 2011). I used the land-cover which had the greatest positive effect on amphibian abundance (forest, for both species, and predicted wetlands, in a second model for southern cricket frogs) and labelled it as “habitat” (resistance = 1). I valued the resistance of all other landscape features through their relationship with “forest” according to the prior model; the landscape features with the most negative effect on amphibian abundance were assigned the highest resistance values (e.g., open water resistance = 30). This was similar to the approach used by others in resistance modeling, in which the resistance layer created is the inverse of habitat suitability models (Zeller et al. 2012). I used the resistance layer with ArcGIS tools Cost Path (Fig. 3.2) to create theoretical least-cost paths of amphibian dispersal across the landscape (Adriaensen et al. 2003) among the study GIWs. I used correlation to examine the relationship between log-transformed F_{ST} and log-transformed cost paths for both southern leopard frog and southern cricket frogs. Results were considered statistically significant if $p < 0.05$. I created two resistance models for southern cricket frogs; the first (hereafter MODEL1) used forest patches as the base, most suitable habitat (resistance=1), whereas the second (hereafter MODEL2) used predicted wetlands as the most suitable habitat (resistance=1). I tested the correlations between log-transformed F_{ST} and log-transformed cost paths.

Lastly, I created models which linked F_{ST} values to the landscape composition between pairwise GIWs. I created corridors between the GIWs (180 m wide, or six 30 m-wide pixels of NLCD raster data) and used grouped NLCD classes to model their predictive effects on population dynamics via gene flow (six classes, Table 3.1). I transformed the proportion of these classes (using arcsine square root transformations, (Ahrens et al. 1990)) within corridors, weighted by Euclidean distance, in the models. I transformed the F_{ST} values using logarithmic transformation or Box Cox transformations (Box and Cox 1964, Osborne 2010) as appropriate.

RESULTS

I processed 288 DNA samples over two field seasons, from May 2015—October 2015 and February 2016 – April 2016. Either southern cricket frog or southern leopard frog populations were supported or analyzed in 24 of 33 study GIWs. I obtained F_{ST} values for the pairwise interactions between each GIW population (Appendix A, Tables 1-3). F_{ST} values ranged from 0.075 to 0.279 for southern cricket frogs, with an average F_{ST} of 0.142 and ranged from 0.073 to 0.860 for southern leopard frogs, with an average F_{ST} of 0.187. For southern cricket frogs, the correlation between F_{ST} and Euclidean distance was positive and statistically significant (R^2=0.364, p=0.003, Fig. 3.3). For southern leopard frogs, the correlation between F_{ST} and Euclidean distance was positive but the relationship was not statistically significant (R^2 = 0.183, p=0.082, Fig. 3.4). Next, I tested the correlations between F_{ST} and cost path distance, hereafter referred to as cost distance. For southern cricket frogs, I had made two resistance layers, with forest as primary habitat in the first and predicted wetlands as the primary habitat in the second; the correlation between F_{ST} and cost distance 1 was 0.200, and the correlation between F_{ST} and cost distance 2 was 0.399 (p= 0.108 and p=0.001, Figs. 3.5 and 3.6, respectively). For southern leopard frogs, the correlation between F_{ST} and cost distance was 0.185 (p=0.080, Fig. 3.7).

Two parameters, forest and agriculture (proportional variables before arcsine square root transformation), were collinear (R^2 = -0.787), so I compared general linear models without forest or agriculture. The genetic differentiation (F_{ST}) of southern cricket frog GIW populations were best explained by a global model, which suggested that agriculture and urban land-use negatively affected F_{ST} for this species, while herbaceous and shrub land-cover had a positive effect on F_{ST} ($p< 0.05$) (Table 3.2). However, southern leopard frog F_{ST} was negatively associated with agriculture and wetland land use ($p < 0.05$) (Table 3.3).

DISCUSSION

I used double-digest restriction site-associated DNA markers (ddRADseq) protocols, which enable genotyping through the automated scoring of single-nucleotide polymorphisms (Davey et al. 2011, Andrews et al. 2016). This method allows reduced-representation sequencing, which results in the cost-effective production of thousands of genetic markers (Arnold et al. 2013). Double-digest RADseq protocols differ from basic RADseq in two aspects: the DNA digestion process uses two restriction enzymes, which makes the creation of a DNA library less expensive, and sequencing from both ends is efficiently targeted to the same beginning nucleotide, which increases coverage per sequencing dollar (Peterson et al. 2012). I used a variant of ddRADseq, 3RAD, which has additional advantages in producing low-cost libraries and facilitates future studies that may use RADcap (Hoffberg et al. 2016).

There was a large disparity in the maximum F_{ST} value of my two species, where maxF_{ST} of southern cricket frogs was 0.279 and maxF_{ST} of southern cricket frogs was 0.860. The discrepancies were mostly a function of the southern leopard frog species' interaction including a GIW population which was separated from the other GIW complexes by a major waterway (i.e., the Flint River.) In addition to these interactions, there was a single high-F_{ST} interaction between the populations of two GIWs. This appears to be an anomaly, as all other interactions involving those GIWs fall within normal bounds.

The interactions between pairwise wetland populations overall followed the expected pattern, although to a lesser degree. Outlying interactions could be a result of non-random mating. Amphibians show high philopatry to their natal wetlands, although this varies regionally and by species (Smith and Green 2006, Semlitsch 2008). Additionally, amphibian competition for mates is high (Gerhardt 1991), and anuran mate choice depends largely on male calling (Arak

1983, Ryan and Keddy-Hector 1992, Tregenza and Wedell 2000). Inbreeding is therefore common in pond-breeding amphibian populations (Shields 1982). This inbreeding could take the form of half-siblings, through multiple paternity of the same clutch, or through male promiscuity, with fathers siring multiple clutches (Smith and Green 2005). Siblings may have been included in analyses, although attempts were made to minimize sibling take; I did not collect tadpole specimens encountered within 5 m of the last tadpole collection, and chose specimens of varying age-class, when possible.

I found a higher degree of variability in F_{ST} values and a lower degree of correlation to distance and cost distance than expected. I initially predicted that F_{ST} would be positively and logarithmically related to both Euclidean distance and cost distance, with measures of cost-distance demonstrating a higher correlation than Euclidean distance. I created two resistance models for southern cricket frogs; the first (hereafter MODEL1) used forest patches as the base, most suitable habitat (resistance=1), whereas the second (hereafter MODEL2) used predicted wetlands as the most suitable habitat (resistance=1). MODEL1 explained far less variation (Fig. 3.5, R^2 = 0.200) than either a Euclidean distance-based model (Fig. 3.3, R^2 = 0.364) or MODEL2 (Fig. 3.6, R^2 = 0.399). The cost-distance model (Fig. 3.7, R^2 = 0.187) and the Euclidean distance model for southern leopard frogs (Fig. 3.4, R^2 = 0.185) were both poor predictors of genetic differentiation.

My results support the contention that southern cricket frogs have a stronger relationship between genetic differentiation and distance than southern leopard frogs. Southern cricket frogs are small-bodied and are likely less vagile than large southern leopard frogs. Their higher surface-area-to-volume ratio could increase the relative amounts of energy required for long dispersal events or migrations (Rittenhouse et al. 2009), and previous literature has indicated that southern cricket frogs are more likely to remain close to wetland edges during non-breeding seasons (Jensen 2008). In contrast, southern leopard frogs are large anurans with relatively high vagility (Seburn et al. 1997, Carr and Fahrig 2001, Lehtinen and Galatowitsch 2001, Smith and Green 2006, Graeter et al. 2008, McKee 2012) and tolerance to environmental stressors (Lindstedt and Boyce 1985), and showed little genetic differentiation even at the largest spatial scale.

Another factor which may explain the higher degree of genetic differentiation among southern cricket frog populations is hinted at by the relatively high correlation of MODEL2, which used "predicted wetlands" as the most suitable and most permeable habitat for this species. Predicted wetlands were delineated based on presence of hydric soils (Martin et al. 2012) and were more extensive than wetland habitat. Amphibians are susceptible to water loss across their permeable skin (Pounds and Crump 1994) and are often associated with hydric soils (Thorson and Svihla 1943, Spight 1967, Wyman 1988, Conant and Collins 1998), since the primary water source of amphibians is environmental moisture (Tracy 1976), especially the potential water of soils (Bury and Corn 1988, Wyman 1988).

In addition to testing resistance layers built from models of amphibian abundance in GIWs (Chapter 2), I modeled the influence of landscape features on genetic differentiation of two amphibian species. The top model for the southern cricket frog suggests that the genetic differentiation was negatively affected by urban land-cover and positively affected by herbaceous land-cover. Low F_{ST} values indicate panmictic populations, whereas high F_{ST} values indicate a greater degree of genetic differentiation and lower levels of gene flow. Thus, urban and agricultural land-cover appeared to facilitate gene flow in southern cricket frogs and herbaceous and shrub land-cover appeared to hinder it. This finding is counter-intuitive, as urban and agricultural land-cover has been linked to negative effects on amphibian presence and abundance (Knutson et al. 1999, Pillsbury and Miller 2008), including of southern cricket frogs (Simon et al. 2009). Herbaceous and shrub land-use in the Dougherty Plain may hinder gene flow by not providing ample cover, forage, and soil moisture for dispersing southern cricket frogs (Cushman 2006, Semlitsch 2008). However, additional data on fine scale vegetation structure in these land uses and agriculture and urban land uses in the region are needed to confirm this. An examination of the composition of least cost paths between wetland pairs may provide more insight into why some populations were more similar than would be expected based on the composition of a 180 m wide Euclidean buffer.

The top model for southern leopard frogs suggests that gene flow is or has been facilitated by agricultural and wetland land-cover. Although most amphibian species are negatively affected by agricultural land-use (Guerry and Hunter 2002), those with large body size (Gray et al. 2004), including southern leopard frogs (Alix et al. 2014), seem to be less affected by agricultural land-cover. Wetland facilitation of gene flow is unsurprising, as wetlands are primary habitat of most amphibians (Semlitsch and Bodie 1998) and because literature suggests amphibians may use wetlands as stepping stones towards new breeding ponds (Amezaga et al. 2002, Spear et al. 2005).

Another possible explanation for the negative relationship between F_{ST} of southern cricket frogs and agricultural and urban land-use is that these fairly recent land-use changes are not reflected in the current populations. Thus, the low F_{ST} values may reflect more frequent gene flow in the past, when landscapes were more permeable. Investigating additional measures of allelic diversity may provide evidence for this hypothesis. Nonetheless, I found significant genetic differentiation of southern cricket frog populations in 24 GIW sites within the Dougherty

Plain. A model incorporating resistance to movement which identified predicted wetlands as primary habitat was better than simple Euclidean distance in explaining this differentiation. Alternatively, southern leopard frogs, a larger and more vagile anuran, did not display significant genetic differentiation among wetlands in this landscape; even our most distant GIWs showed only moderate differentiation when not separated by a major river (distance = 44,749 m; F_{ST} value = 0.40). It should be noted that amphibians are unlikely to move exclusively in straight lines between wetlands; in future work, I hope to use F_{ST} values to test parameters of landscape resistance to explore which view of the landscape best fits the observed patterns of genetic differentiation.

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Table 3.1. National Land Cover Data (NLCD 2011) classifications used to develop amphibian models in the Dougherty Plain in southwestern Georgia, 2015-2016.

Combined Classification	Code	NLCD
Open Water	11	Open Water
Wetlands	91	Woody Wetlands
	92	Emergent Herbaceous Wetlands
Urban	21	Developed Open Space
	22	Developed Low Intensity
	23	Developed Medium Intensity
	24	Developed High Intensity
Forest	41	Deciduous Forest
	42	Evergreen Forest
	43	Mixed Forest
Herbaceous	52	Shrub / Scrub
	71	Grassland / Herbaceous
	72	Sedge / Herbaceous
Agriculture	81	Pasture / Hay
	82	Row Crops
	85	Urban / Recreational Grasses

Table 3.2. Models explaining the genetic differentiation (F_{ST}) of southern cricket frogs between populations in geographically isolated wetlands using the land-use in the 180 m wide corridors between them. Classes include open water, wetlands, forest, agriculture, urban, and herbaceous cover. Observations, log likelihood, and Akaike's Information Criterion corrected for small sample sizes (AICc) are given for both models. Estimates include 95% confidence intervals in parentheses.

	Dependent variable:	
	Adjusted FST for southern cricket frogs	
Constant	0.59 (-0.23, 1.42)	1.49*** (0.90, 2.08)
water	0.82 (-0.70, 2.33)	0.03 (-1.49, 1.56)
forest	0.42 (-0.17, 1.01)	
wetlands	-0.13 (-0.80, 0.54)	-0.42 (-1.06, 0.22)
urban	-2.37** (-4.14, -0.60)	-1.77** (-3.48, -0.05)
ag		-0.81*** (-1.31, -0.30)
herb	1.20*** (0.45, 1.94)	0.72** (0.04, 1.39)
Observations	66	66
Log Likelihood	-26.59	-22.63
AIC_C	65.18	57.25
Note:		$^{*}p<0.1$; $^{**}p<0.05$; $^{***}p<0.01$

Table 3.3. Models explaining the genetic differentiation (F_{ST}) of southern leopard frogs between populations in geographically isolated wetlands using the land-use in the corridors between them. Classes include open water, wetlands, forest, agriculture, urban, and herbaceous cover. Observations, log likelihood, and Akaike's Information Criterion corrected for small sample sizes (AICc) are given for both models. Estimates include 95% confidence intervals in parentheses.

	Dependent variable:	
	Adjusted FST for southern leopard frogs	
Constant	0.23^{**} (0.04, 0.42)	0.38^{***} (0.26, 0.49)
water	-0.08 (-0.35, 0.20)	-0.16 (-0.43, 0.11)
forest	0.09 (-0.04, 0.22)	
wetlands	-0.13 (-0.31, 0.05)	-0.21^{**} (-0.39, -0.03)
urban	-0.44^{**} (-0.83, -0.06)	-0.31 (-0.69, 0.06)
ag		-0.15^{***} (-0.24, -0.06)
herb	0.09 (-0.10, 0.28)	0.07 (-0.08, 0.22)
Observations	91	91
Log Likelihood	85.12	89.23
AIC_C	-158.23	-166.47
Note:		$^{*}p<0.1$; $^{**}p<0.05$; $^{***}p<0.01$

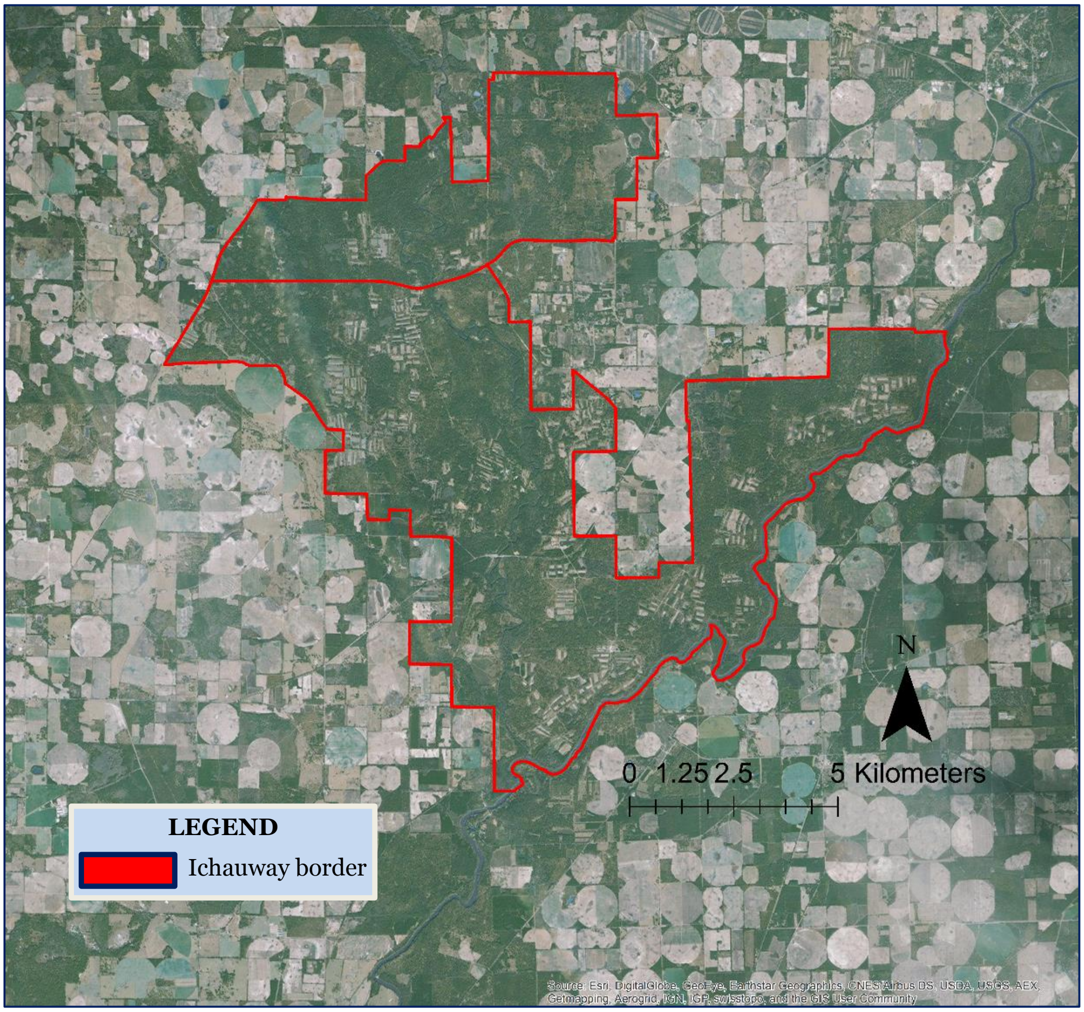

Fig. 3.1. An aerial view of a portion of the Dougherty Plain, southwestern Georgia, with Ichauway, the research site of the Joseph W. Jones Ecological Research Center outlined in red.

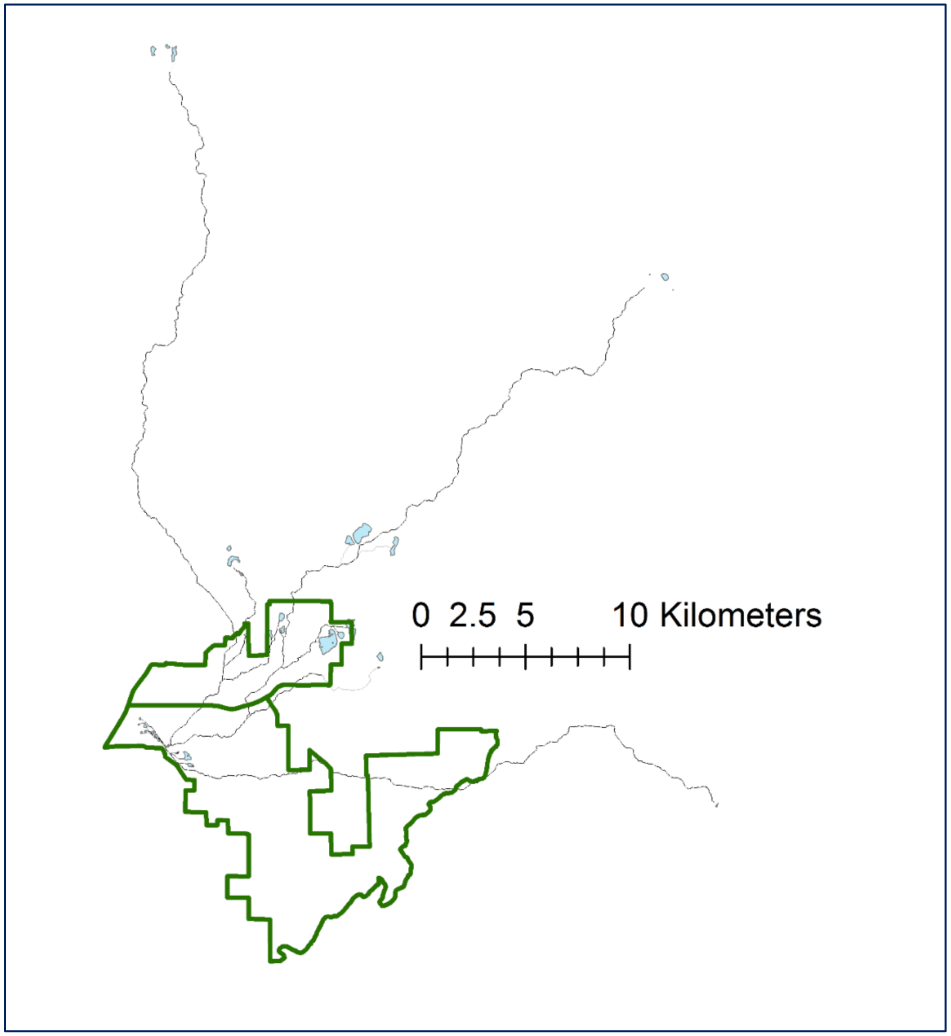

Fig. 3.2. An example of the least-cost paths between geographically isolated wetlands for amphibians created through application of cost-distance resistance layers in southwestern Georgia in 2015-2016.

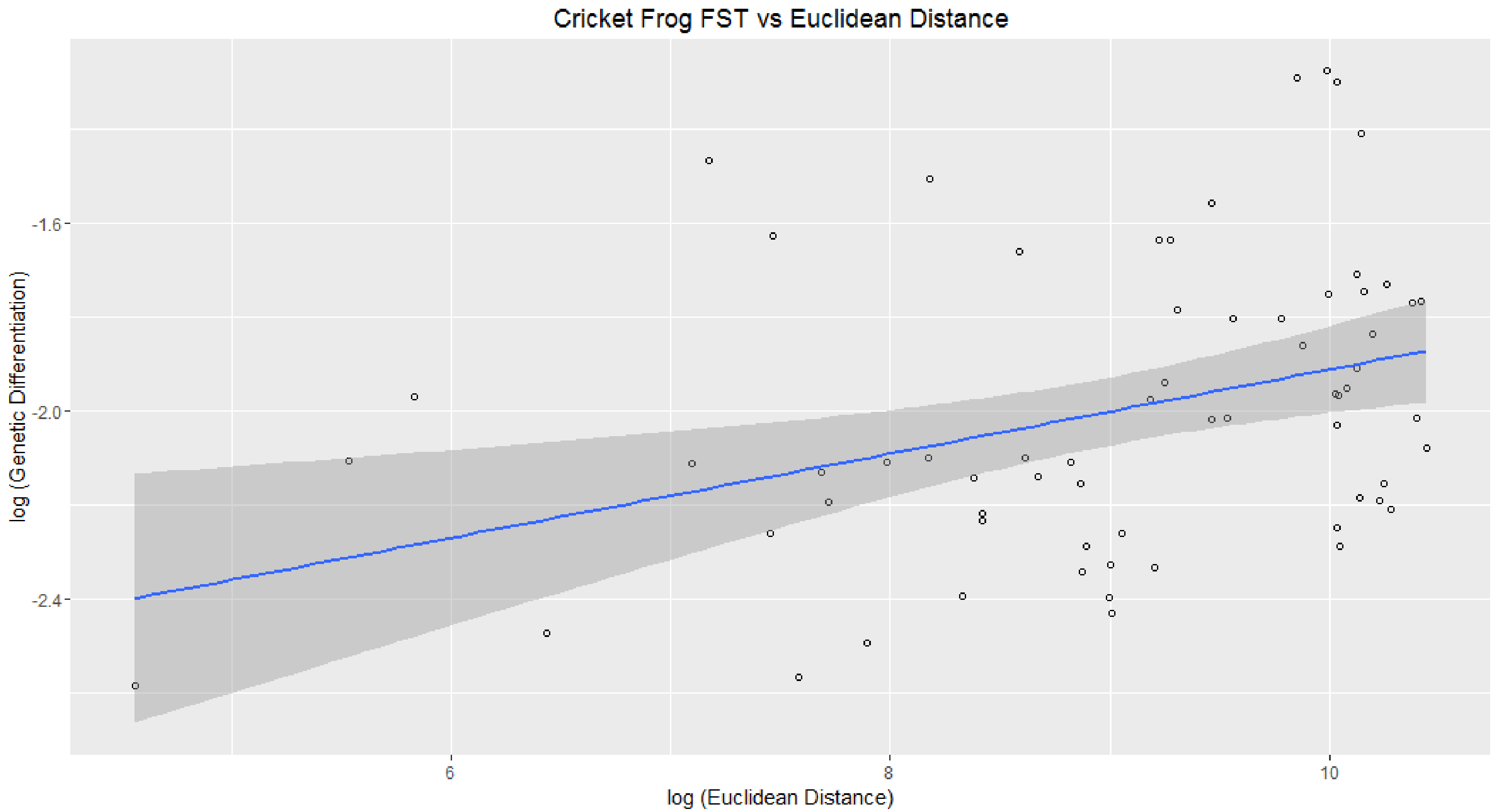

Fig. 3.3. Relationship (R^2= 0.364, p = 0.003) between the log of Euclidean distance between pairs of GIWs (x-axis) and the log of genetic differentiation (F_{ST}; y-axis) for southern cricket frogs in southwestern Georgia in 2015-2016.

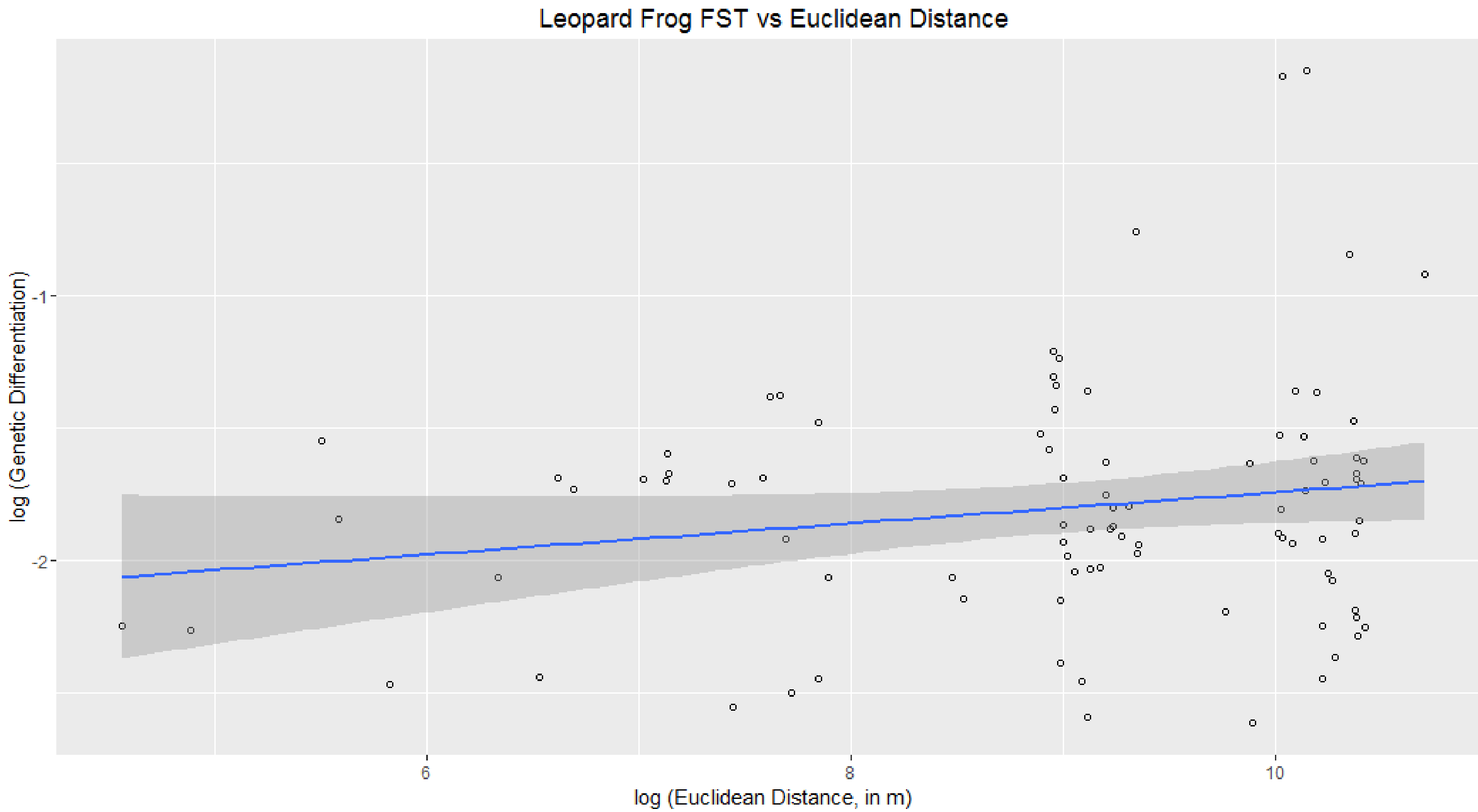

Fig. 3.4. Relationship (R^2= 0.183, p = 0.082) between the log of Euclidean distance between pairs of GIWs (x-axis) and genetic differentiation (F_{ST}; y-axis) for southern leopard frogs in southwestern Georgia in 2015-2016.

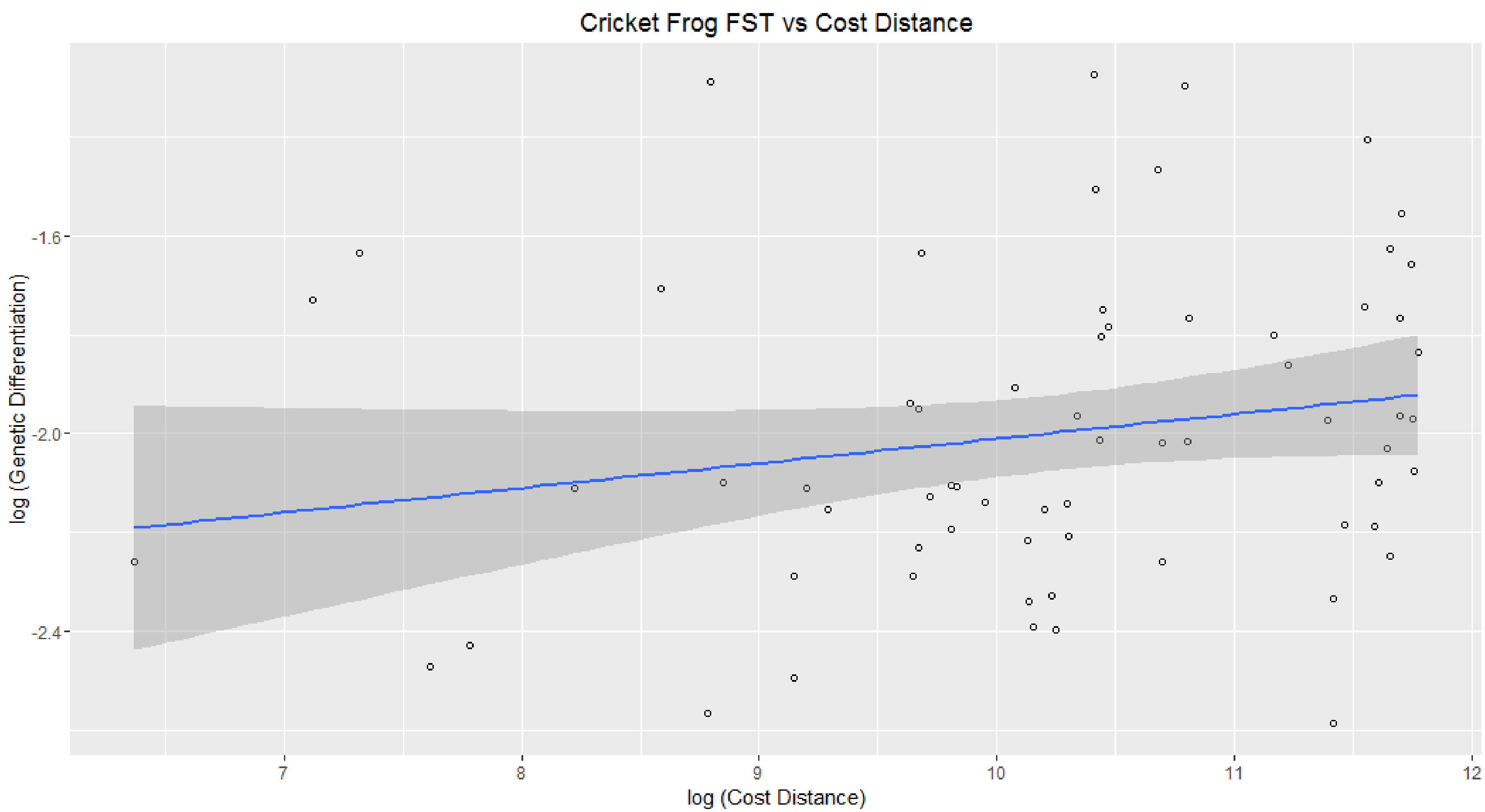

Fig. 3.5. The correlation of MODEL 1 cost-distance and F_{ST} (R^2= 0.200, p = 0.108) for southern cricket frogs in the Dougherty Plain, Southwestern Georgia. This model considered forest the most suitable (permeable) habitat for southern cricket frogs in southwestern Georgia in 2015-2016.

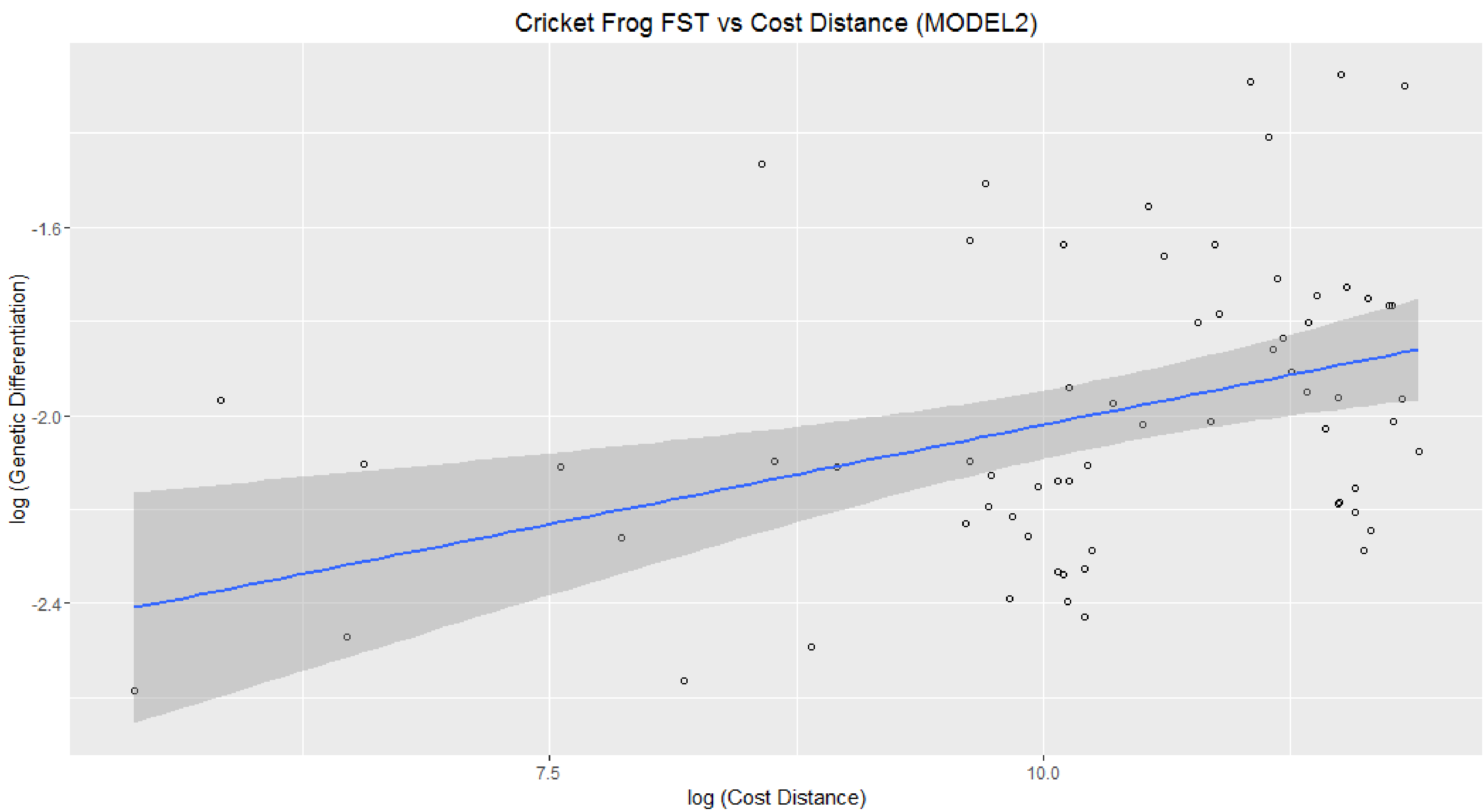

Fig. 3.6. The correlation of MODEL 2 cost-distance and F_{ST} (R^2= 0.399, p = 0.001) for southern cricket frogs in the Dougherty Plain, southwestern, Georgia. This model used predicted wetlands as the most suitable (permeable) habitat for cricket frogs in southwestern Georgia in 2015-2016.

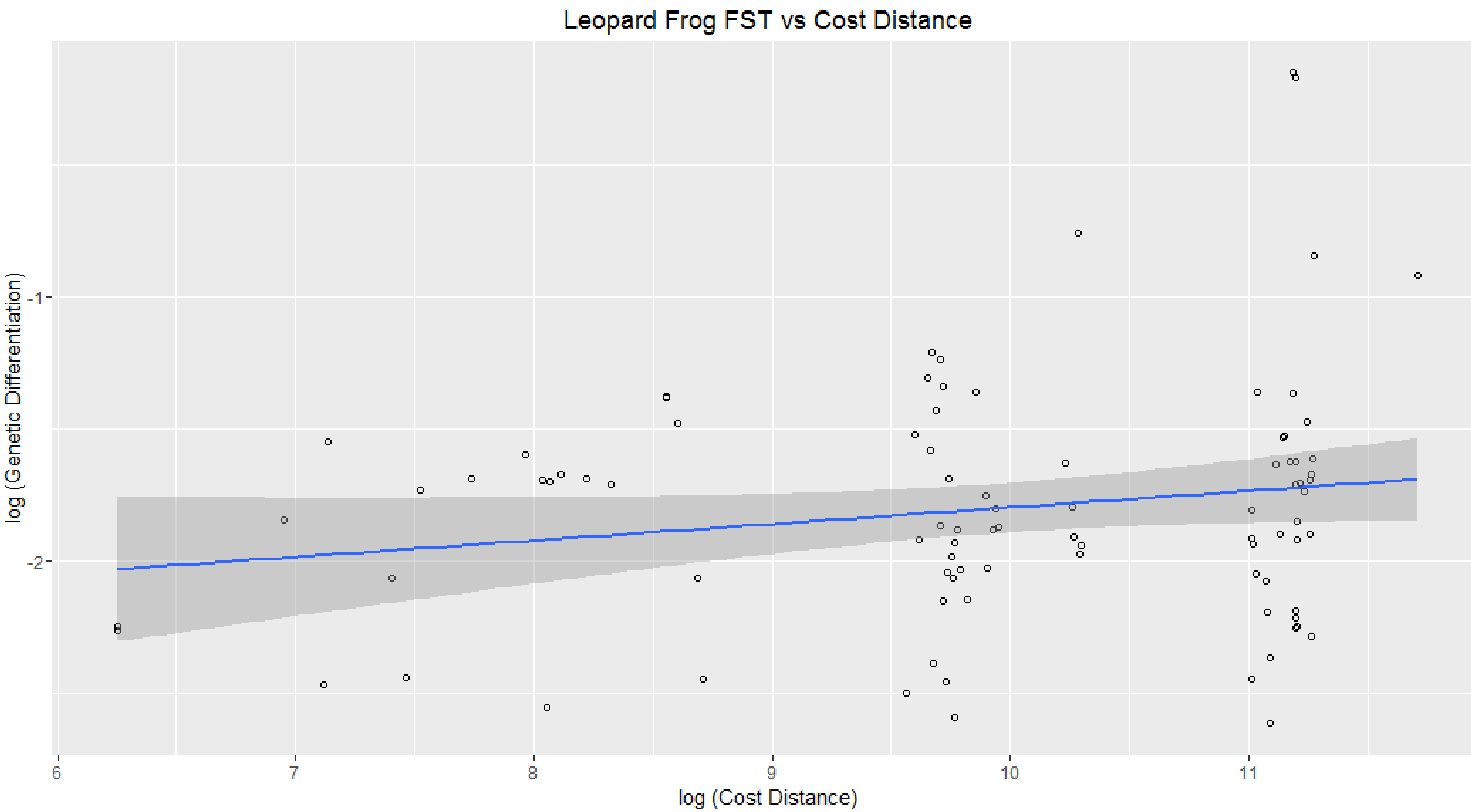

Fig. 3.7. The correlation of the least cost distance and F_{ST} (R^2= 0.184, p = 0.080) for southern leopard frogs in southwestern Georgia in 2015-2016.

CHAPTER 4

CONCLUSIONS

The scientific consensus is that amphibians are imperiled (Gibbons et al. 2000, Hoffmann et al. 2010); about a third of amphibian species are threatened (Stuart et al. 2004), and extinction rates of amphibians are more than 200 times the background rate (McCallum 2007). This pattern is as true of the United States as it is globally (Lannoo 2005), with 36 of the 300 species in the U.S. recognized as “critically imperiled” or “presumed to be extinct” (Natureserve 2011). Despite the acknowledgement of widespread amphibian loss, mitigation of threats has proven difficult (Mendelson et al. 2006). Amphibian populations are more threatened than both birds and mammals (Stuart et al. 2004), but receive far less funding; the U.S. Fish and Wildlife Service (FWS) spent only 2.7% of their recovery funding on amphibians in 2009. This taxonomical bias (Gratwicke et al. 2012) toward more charismatic animals (Gunnthorsdottir 2001) extends to public opinion and increased private fiscal support (Kellert 1985, Kotchen and Reiling 2000).

The decline of amphibian populations is in large part due to anthropogenic habitat conversion; growing industry, large-scale agriculture, and sprawling urban centers (Vitousek et al. 1997, Foley et al. 2005). These landscape alterations cause habitat loss, degradation, and fragmentation (Brooks et al. 2002, Fahrig 2003, Cushman 2006). These effects may be particularly detrimental to amphibians, whose small body size and complex natural histories render them increasingly vulnerable to habitat shifts (Semlitsch and Bodie 2003, Cushman 2006). If amphibians are declining, and they are particularly vulnerable to habitat loss and fragmentation, then to protect them we must stop habitat conversion or mitigate its effects.

Because human development is unlikely to slow, instead, managers should determine which habitats should be prioritized for preservation.

Despite the need for the information to support these conservation and management plans, studies which address species- and assemblage-specific effects of landscape composition and configuration on multiple spatial scales remain rare (Cushman 2006, Veysey et al. 2011). This is partially due to the difficulty of separating local effects from the effects of landscape-scale features (Hamer and McDonnell 2008). In addition, studies which address amphibian natural histories have declined drastically (McCallum and McCallum 2006), so researchers often must rely on anecdotal evidence to estimate baseline amphibian habitat suitability and movement. Policymakers may believe that to receive funding, best plans of management should be scientifically supported and data-driven—but how can they be, when the salient information has not been garnered?

To contribute to these information deficits and determine the effects of landscape features on amphibians, I studied amphibian abundance and community composition, as well as amphibian gene flow in geographically isolated wetlands on the Dougherty Plain of GA, USA. I surveyed 33 geographically isolated wetlands (GIWs) to determine the presence and abundance of breeding amphibian species, and modeled the effects of landscape features on the abundance of selected species (i.e., southern leopard frogs, southern cricket frogs, ornate chorus frogs, and longleaf pine specialist species) on several spatial scales (i.e., 100 m, 250 m, 500 m, and 1 km).

The effects of landscape features did not affect abundance of amphibian species equally. For southern leopard frogs only marsh wetland vegetation had a significant positive effects on abundance. Southern cricket frogs, ornate chorus frogs, and longleaf pine specialists as a guild, however, were positively correlated with the amount of forest cover in the uplands surrounding

GIWs. Forest cover has identified in the literature as a principal indicator of local amphibian presence and biodiversity (Guerry and Hunter 2002, Werner et al. 2007, Farmer et al. 2009). Longleaf pine specialist species and longleaf residents may depend on open-canopy forests with herbaceous undergrowth (Guyer and Bailey 1993) due to evolutionary associations with the pyrogenic longleaf pine ecosystem (Steen et al. 2010); furthermore, previous literature has linked ornate chorus frogs (Todd et al. 2009) and southern cricket frogs (Knutson et al. 2000) to intact, forested landscapes. "Predicted wetlands" were positively correlated with southern cricket frog and ornate chorus frog abundance. These predicted wetlands were delineated based on hydric soils with high moisture content (Martin et al. 2012), an abiotic factor which has been linked to amphibian presence in other studies (Thorson and Svihla 1943, Wyman 1988, Conant and Collins 1998, Gibbons 2003). My results suggest that amphibians may benefit from the protection of GIWs embedded in forest patches and in landscapes with areas of hydric soils, although amphibian response to these landscape features were far from uniform.

My examination of landscape connectivity via genetic differentiation afforded similar results. I examined the gene flow of southern cricket frogs and southern leopard frogs across the landscape, first using F_{ST} values to test landscape resistance layers, and then using genetic differentiation (F_{ST} values) to model the impacts of land-cover. Euclidean distance, cost distance, and models were inadequate to explain the variation in genetic differentiation or abundance of southern leopard frogs. This is most likely due to their large body size, high vagility, and high tolerance to environmental stressors (Lindstedt and Boyce 1985). However, misspecification of resistance values may have also resulted in poor correlations with values of genetic differentiation between populations. Conversely, landscape features had distinct effects on the abundance and genetic differentiation of the smaller southern cricket frog. Southern cricket frog genetic differentiation was significantly and positively correlated with Euclidean distance, and a resistance layer incorporating predicted wetlands as primary cricket frog habitat and assigning resistance to movement weights according to importance in determining local abundance improved model fit. This supports the assumption that proximity to hydric soil is vital to the survival of this small-bodied anuran, potentially due to their vulnerability to desiccation.

Overall, my study suggests that southern cricket frogs are more dependent on landscape features than southern leopard frogs; moreover, to preserve amphibians, conservation efforts should be focused on the preservation of wetlands, especially those wetlands in proximity to forests or predicted wetlands. However, these questions are far from adequately answered; in the future, additional analyses of more explicit least-cost path functions including hydrological features such as intermittent flow paths should be considered. I hope to create a model that provides reasonable landscape resistance values that align with observed F_{ST} values. To achieve this objective, I plan to consider different models of amphibian movement, including those that simultaneously account for several potential amphibian paths between GIWs and those that consider amphibian movement as a series of decisions across the landscape.

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APPENDIX A

GEOGRAPHICALLY ISOLATED WETLAND SAMPLING DATA, 2015-2016

Appendix A contains tables that connect the identification of my study wetlands to the UTM, Zone 16, NAD 1983 XY coordinates at which they may be located (Table A.1), as well as tables which summarize the pairwise F_{ST} values of southern cricket frog populations (Table A.2) and southern leopard frog populations (Table A.3).

Table A.1. UTM coordinates of geographically isolated wetlands sampled for amphibians in the Dougherty Plain of southwestern Georgia in 2015-2016.

OS_ID	X COORDINATE	Y COORDINATE
W12	740610.04	3466074.58
W00	741153.06	3466871.19
W46	736636.61	3460110.85
W13	741177.07	3466251.05
W21	743998.73	3466001.00
W42	735694.62	3460684.22
W49	736787.62	3459771.85
W23	744477.12	3466329.09
W37	734632.23	3461397.87
W41	735266.13	3460967.83
W35	734374.99	3461997.50
W96	734311.52	3461716.79
W36	734618.02	3461654.25
W97	734428.91	3461333.00
W39	734974.67	3461339.22
W40	735139.10	3461170.02
W44	736152.25	3460350.72
W20	743385.93	3465654.57
REDRH	745800.29	3464453.19
CN3	746656.40	3470492.18
CN1	744406.40	3470520.42
CN2	744990.72	3470898.07
EL3	739195.23	3468937.63
EL2	738853.50	3469699.44
AZD	736012.42	3493840.23
AZ	734987.02	3494000.88
AZR	735702.48	3494167.97
PN3	759904.01	3482539.43
PN1	758789.73	3483285.28
PN2	759523.23	3483161.85
EL1	738636.10	3470159.32
RB2	761999.66	3457830.07
RB1	762058.39	3457943.01
REDRR	745883.47	3464953.78

Table A.2. Indices of genetic differentiation (F_{ST}) for pairwise comparisons of geographically isolated wetlands for populations of southern cricket frogs in the Dougherty Plain of southwestern Georgia in 2015 and 2016 (1 of 2).

Paired GIWs	F_{ST}
W00_AZR	0.112068
W00_CN1	0.107392
W00_CN3	0.117787
W00_N1	0.142329
W00_W12	0.084505
W00_W20	0.07689
W00_W21	0.082606
W00_W23	0.121351
W00_W41	0.097673
W00_W49	0.0911
W12_AZR	0.116041
W12_CN1	0.122749
W12_CN3	0.121463
W12_N1	0.148387
W12_N3	0.112565
W12_W23	0.122674
W12_W41	0.101632
W12_W42	0.116122
W12_W49	0.096338
W20_CN1	0.091525
W20_CN3	0.109025
W20_N1	0.131435
W20_N3	0.101466
W20_REDRH	0.1115
W20_W21	0.075426
W20_W42	0.104563
W20_W49	0.088157
W21_AZR	0.109973
W21_CN3	0.117611
W21_N1	0.140431
W21_N3	0.105744
W21_REDRH	0.119069
W21_W23	0.12193
W21_W41	0.097035

Table A.2. Indices of genetic differentiation (F_{ST}) for pairwise comparisons of geographically isolated wetlands for populations of southern cricket frogs in the Dougherty Plain of southwestern Georgia in 2015 and 2016 (2 of 2).

Paired GIWs	**F_{ST}**
W23_AZR	0.177535
W23_CN1	0.221682
W23_N1	0.278995
W23_N3	0.173655
W23_REDRH	0.196412
W23_W41	0.143734
W23_W42	0.194755
W23_W49	0.138863
W41_CN3	0.164834
W41_N3	0.133537
W41_REDRH	0.168069
W41_W42	0.139645
W41_W49	0.10444
W42_AZR	0.170799
W42_CN1	0.211102
W42_REDRH	0.194645
W42_W49	0.121216
W49_AZR	0.125311
W49_CN1	0.1329
W49_CN3	0.133386
W49_N1	0.17076
AZR_CN1	0.181154
AZR_CN3	0.174793
AZR_N1	0.244655
AZR_N3	0.159464
CN1_N1	0.275008
CN1_N3	0.155535
CN3_N3	0.165125
CN3_REDRH	0.190212
N1_N3	0.230765
N1_REDRH	0.273048
N3_REDRH	0.140198

Table A.3. Indices of genetic differentiation (F_{ST}) for pairwise comparisons of geographically isolated wetlands for populations of southern leopard frogs in the Dougherty Plain of southwestern Georgia in 2015 and 2016 (1 of 3).

Paired GIWs	**F_{ST}**
W00_AZR	0.086735
W00_N1	0.144783
W00_RB1	0.217301
W00_REDRH	0.117004
W00_W13	0.213172
W00_W20	0.185263
W00_W21	0.127126
W00_W35	0.145129
W00_W36	0.185244
W00_W37	0.137532
W00_W41	0.155461
W00_W42	0.116446
W00_W46	0.29948
W13_AZR	0.129474
W13_N1	0.25706
W13_RB1	0.149865
W13_REDRH	0.127322
W13_W20	0.077794
W13_W21	0.228153
W13_W35	0.262731
W13_W36	0.23961
W13_W37	0.291374
W13_W41	0.270684
W13_W42	0.206521
W13_W46	0.218984
W20_AZR	0.125545
W20_N1	0.147711
W20_RB1	0.07349
W20_REDRH	0.082057
W20_W21	0.105746
W20_W35	0.13103
W20_W36	0.074909
W20_W37	0.152406
W20_W41	0.085654

Table A.3. Indices of genetic differentiation (F_{ST}) for pairwise comparisons of geographically isolated wetlands for populations of southern leopard frogs in the Dougherty Plain of southwestern Georgia in 2015 and 2016 (2 of 3).

Paired GIWs	F_{ST}
W20_W42	0.129566
W20_W46	0.092054
W21_AZR	0.094156
W21_N1	0.164516
W21_RB1	0.195297
W21_REDRH	0.147343
W21_W35	0.154456
W21_W36	0.152478
W21_W37	0.165148
W21_W41	0.174121
W21_W42	0.131727
W21_W46	0.257002
W35_AZR	0.112364
W35_N1	0.199906
W35_RB1	0.182663
W35_REDRH	0.144253
W35_W36	0.158321
W35_W37	0.12745
W35_W41	0.202739
W35_W42	0.180952
W35_W46	0.086942
W36_AZR	0.109448
W36_N1	0.188194
W36_RB1	0.14675
W36_REDRH	0.46849
W36_W37	0.104461
W36_W41	0.17771
W36_W42	0.183533
W36_W46	0.253054
W37_AZR	0.157962
W37_N1	0.101864
W37_RB1	0.106129
W37_REDRH	0.139612
W37_W41	0.087434

Table A.3. Indices of genetic differentiation (F_{ST}) for pairwise comparisons of geographically isolated wetlands for populations of southern leopard frogs in the Dougherty Plain of southwestern Georgia in 2015 and 2016 (3 of 3).

Paired GIWs	**F_{ST}**
W37_W42	0.184659
W37_W46	0.251931
W41_AZR	0.181174
W41_N1	0.18429
W41_RB1	0.256096
W41_REDRH	0.166141
W41_W42	0.085162
W41_W46	0.188119
W42_AZR	0.197546
W42_N1	0.149976
W42_RB1	0.19736
W42_REDRH	0.148488
W42_W46	0.184946
W46_AZR	0.105069
W46_N1	0.2297
W46_RB1	0.216213
W46_REDRH	0.196539
AZR_N1	0.176733
AZR_RB1	0.399204
AZR_REDRH	0.430512
N1_RB1	0.859649
N1_REDRH	0.844444
RB1_REDRH	0.112047